W-PM-A1 THE REACTIVE SULFHYDRYL GROUPS SH_1 AND SH_2 ARE CLOSE TOGETHER IN THE PRIMARY STRUCTURE OF MYOSIN. Marshall Elzinga, Boston Biomedical Research Institute and Harvard Medical School, Boston, Mass. 02114.

As part of a study of the complete amino acid sequence of the heavy chain of rabbit skeletal muscle myosin, a cyanogen bromide peptide of molecular weight about 9,000 has been isolated by gel filtration and ion-exchange chromatography. It contains proline as well as the single residue of N^{τ}-methyl histidine, and must therefore arise from the head portion of the molecule. Partial sequence analysis of this peptide indicates that the sequence, starting from the NH_2-terminus, is: Glu-His-Glu-Leu-Val-Leu-His-Gln-Leu-Arg-Cys-Asn-Gly-Val-Leu-Glu-Gly-Ile-Arg-Ile-Cys-Arg-. A comparison of this sequence with peptides that contain the cysteines which are thought to represent the reactive sulfhydryl groups "SH_1" and "SH_2" (Yamashita et al, J. Biochem.75,447 (1974)) indicates that the residue written Cys is SH_1 and the residue designated Cys is SH_2. Thus these two sidechains, whose alkylation alters the enzymatic properties of myosin, are only 11 residues apart in the sequence, and must be close together in the functioning molecule. Based upon studies that involved chemical crosslinking of sulfhydryl groups in myosin, Reisler et al (Biochemistry 13,3837 (1974)) estimated that these two sulfhydryl groups are 12-14 A apart in native myosin. If the sequence shown above is an α-helix, the two cysteines would be about 16 A apart, while if the peptide assumes another configuration, a slight folding back of the peptide would bring the sidechains into the predicted proximity. As might be expected of a site that is involved in nucleotide binding, the region around these residues is basic and rich in hydrophobic residues; the presence of N^{τ}-methyl histidine in this peptide suggests that this unusual residue is located near (one of) the nucleotide binding sites of myosin. (Supported by NIH grant #HL-17464.)

W-PM-A2 MODELS FOR THE MORPHOLOGY OF SUBFRAGMENT-1 OF MYOSIN. K.M. Kretzschmar*, R.A. Mendelson and M.F. Morales. C.V.R.I., U.C.S.F., San Francisco, CA. 94143.

Previous measurements of X-ray scattering by solutions of myosin subfragment-1 (S1) suggested a radius of gyration of 3.2 nm (Biophys. J. 16, 126a, 1976). By reducing parasitic scattering and employing a position sensitive detector, exposure time has been reduced considerably and the range of angles over which accurate measurements may be made has been extended. Results, using solutions of bovine serum albumin, are in excellent agreement with published data (Anderegg, Beeman, Shulman, and Kaesberg; J. Am. Chem. Soc. 77, 2927, 1955). Measurements at very small angles confirm that the value of the radius of gyration of S1 is 3.2 nm. If S1 is assumed to be an ellipsoid of revolution, of uniform electron density, with a molecular weight of 1.15×10^5 and a partial specific volume of 0.73 cm^3gm^{-1}; then the axial ratio of the ellipsoid is 2.8 (for a prolate ellipsoid) or 0.27 (for an oblate ellipsoid), and the major axis is 13 nm long (prolate) or 10 nm long (oblate). Measurements at larger angles allow models of S-1 morphology to be tested: theoretical scattering curves for various ellipsoids of revolution were calculated and convoluted to account for X-ray beam and detector dimensions. The observed scattering could be approximated by the scattering from ellipsoids with axial ratios of 2.0 to 2.8 (prolate), or 0.27 to 0.37 (oblate). Thus analysis of two parts of the scattering curve, by independent methods, indicate the S1 is non-sperical, and that its morphology may be approximated by ellipsoids of the dimensions given above. More detailed modelling, to specify the shape and size of the S1 molecule more precisely, is in progress. (KMK is a Science Research Council Fellow. Work performed at SSRP, Stanford Linear Accelerator. Supported by USPHS Grant HL-16683, NSF PCM 75-22698 and DMR73-07692.)

W-PM-A3 ANTIBODY SPECIFIC FOR THE A1 LIGHT CHAIN OF SKELETAL MYOSIN. J.C. Holt*, L. Silberstein*, and S. Lowey, Rosenstiel Center, Brandeis University, Waltham, MA 02154

Antiserum to the A1 light chain of myosin (mol. wt. 21,000) contains two populations of specific antibody: One is directed towards the portion of the molecule which is almost completely homologous with the smaller A2 light chain (mol. wt. 17,000), while the other is specific for the 41-residue difference peptide unique to A1. Only the latter difference antibody can therefore be used to distinguish between A1 and A2 (Holt and Lowey, Biochemistry (1975) 14, 4600-4609). We have isolated this fraction from anti-A1 serum as the specific antibody remaining unbound after repeated passage of the serum through columns of Sepharose-coupled A2. Less than 5% of the antibody so obtained was reactive with A2, as judged by direct binding radioimmunoassay. Difference antibody coupled to Sepharose as an immunoadsorbent was, moreover, capable of fractionating heavy meromyosin into two populations, one containing mainly A1, and the other mainly A2. This result not only confirms the specificity of the difference antibody for A1, but also provides the first direct evidence for the existence of myosin isoenzymes. Preliminary results show that fluorescein-labeled difference antibody binds to myosin filaments in myofibrils and in cryostat sections of chicken pectoralis muscle (Gauthier, personal communication). The question of how A1 is distributed in muscle fibers can thus be approached using this highly specific immunological marker.

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W-PM-A4 CALCIUM INDUCED AGGREGATION OF MYOSIN SUBFRAGMENTS. S. S. Margossian and S. Lowey, Rosenstiel Center, Brandeis University, Waltham, MA. 02154.

In the course of measuring the association of HMM and S1 to actin (Fed. Proc. (1976) 35, 1580), we have observed a reduction in affinity constant upon the addition of 0.1mM Ca^{2+} to our basic buffer (0.10M KCl, 10mM imidazole, pH 7.0, 5mM KP_i, 1mM $MgCl_2$). This response to calcium appeared to derive from S1 rather than actin, since the effect depended on the method of S1 preparation. Papain digestion of myosin in 2mM $MgCl_2$ produced a subfragment (Mg·S1) responsive to calcium, whereas digestion in EDTA resulted in a calcium insensitive fragment (EDTA·S1). The only major difference between these subfragments was the presence of intact DTNB light chain in Mg·S1 (Nature (1975) 258, 163). These results suggest that the DTNB light chain is involved in the binding of calcium to vertebrate skeletal myosin. Preliminary hydrodynamic measurements indicate that both Mg·S1 and HMM undergo a conformational change in the presence of calcium: their intrinsic sedimentation coefficients increase by about 10% when 0.1mM Ca^{2+} is substituted for EGTA in the basic buffer. This change appears to arise from a limited aggregation of S1 in calcium as evidenced by the upward curvature of ln c vs. r^2 plots in sedimentation equilibrium experiments. The same material in EGTA is relatively homogeneous in molecular weight. These effects are reversible as demonstrated by the dissociation of the aggregates upon removal of calcium. We conclude that the diminished binding of myosin to actin in calcium is probably caused by a reversible aggregation of myosin heads. Supported by grants from NIH (AM-17350), NSF (GB38203) and MDA. S.S.M. is an Established Investigator of the American Heart Association.

W-PM-A5 EFFECTS OF TEMPERATURE ON THE ACTIVATION VOLUME OF MYOSIN ATPase. Ronald J. Lukasiewicz and Paul Dreizen. Department of Medicine and Program in Biophysics, State University of New York Downstate Medical Center, Brooklyn, N.Y. 11203.

Studies were conducted on the effects of pressure on myosin ATPase at different temperatures from 5° to 25°. These data yield values of activation volume, $\Delta V\ddagger$, which may be related to the rate limiting step during steady state hydrolysis of ATP, and might thus be expected to provide insight into the thermal dependence of the reaction mechanism for myosin ATPase. At 25°, values of $\Delta V\ddagger$ are approximately -33 cc/mole for Ca ATPase and Mg ATPase of myosin and heavy meromyosin (HMM) at KCl concentrations from 0 to 0.5M. Thus, values of $\Delta V\ddagger$ for myosin ATPase are not significantly affected by ionic strength, formation of myosin filaments, or limited proteolysis to HMM. The results are consistent with a rate limiting step, at 25°, which involves release of ATP hydrolytic products from myosin. At 5°, Ca-ATPase of myosin and HMM exhibits values for $\Delta V\ddagger$ of -20 cc/mole in 0.5M KCl and 0 in 0 to 0. 1M KCl. The results indicate a more complex interpretation for the kinetic mechanism at 5°, than at 25°, consistant with the rate limiting step being release of ADP at high ionic strength, and isomerization of myosin-product complexes at low ionic strength. K-EDTA ATPase of myosin is characterized by values for $\Delta V\ddagger$ of approximately 0 cc/mole at 5° and 25°, under a variety of conditions, consistent with a rate limiting step involving isomerization or bond hydrolysis prior to product release. These results clarify some of the earlier controversy concerning pre-steady state kinetics for myosin ATPase, suggesting that variant interpretations may reflect the thermal conditions under which different assays were done. Supported by USPHS grants AM-06165 and HL-16596.

W-PM-A6 THE Mg^{2+}-ADENOSINE TRIPHOSPHATASE ACTIVITY OF SKELETAL MUSCLE MYOSIN. L.H. Schliselfeld, Department of Biological Chemistry, University of Illinois at the Medical Center, Chicago, Illinois 60612.

The Mg^{2+}-ATPase activity of skeletal muscle myosin was studied at low ionic strength, pH 7.4, and 25°. The activity follows a biphasic curve with respect to ATP concentration. In the concentration range of 0.10-1.0 μM ATP the activity followed normal Michaelis-Menten kinetics with a K_m of 0.4 μM and a V_{max} of 6.9 nmoles P_i/min per mg myosin. As the ATP concentration rose up to 153 μM the activity almost doubled to give an apparent K_m of 10 μM and a final V_{max} of 13 nmoles P_i/min per mg myosin. No biphasic curve is found for myosin Mg^{2+}-ATPase in concentrated NaCl and for heavy meromyosin Mg^{2+}-ATPase at low ionic strength. The high affinity K_m for myosin Mg^{2+}-ATPase activity varies between 0.2 and 0.4 μM in the pH range of 9.0 to 6.5. As the pH drops from 6.5 down to 5.96 the K_m rises to 1.6 μM. This suggests that an ionizable group on either myosin or ATP with a pK_a of about 6.5 is involved in the binding of ATP to the protein. (Supported by Grants from the Public Health Service No. HL 18179-02, the Chicago Heart Association and the Muscular Dystrophy Association, Inc.).

W-PM-A7 FLUORESCENCE STUDIES OF THE INTERACTION OF 1,N^6-ETHENOADENOSINE NUCLEOTIDES WITH ENZYMATIC MYOSIN FRAGMENTS USING SINGLE PHOTON FLUORIMETRY AND STOPPED FLOW KINETICS. Frank Garland and Herbert C. Cheung, Department of Biomathematics, Biophysics Section, University of Alabama in Birmingham, Birmingham, AL 35294.

The two nucleotide binding sites of myosin were studied by monitoring the interaction of 1,N^6-ethenoadenosine 5'-diphosphate (ε-ADP) and triphosphate (ε-ATP) with both heavy meromyosin (HMM) and myosin subfragment-1 (S-1), using single photon fluorimetry and stopped flow kinetics. The single photon experiments show that the excited state lifetime of ε-ADP decreases from 27.2 ± 0.2 nsec to 23.0 ± 0.4 nsec upon binding to S-1 in the absence of Mg^{++}. In the presence of Mg^{++} the decay data exhibits biexponential behavior, with τ_1 = 9 ± 3 nsec and τ_2 = 22.1 ± 0.7 nsec. These data suggest the existence of two bound ligand states with Mg^{++} playing a key role in the transition between the two states. Stopped flow kinetic experiments, in which intrinsic protein fluorescence was monitored, yielded an association rate constant which is in agreement with previously published results. Further experiments, in which complex formation was monitored via protein→ligand energy transfer or direct ligand excitation, show a biphasic trace for the interaction of either ε-ATP or ε-ADP with either HMM or S-1. Computer analysis of the digitized traces of ε-ADP + S-1 indicate that the initial rapid phase is second order with respect to the reactants and that the slow phase is first order with an apparent rate constant of 0.03 sec^{-1}. These results (a) demonstrate a two (or more) step process for the association of the protein with ε-ADP; (b) suggest a similar process for the binding of ε-ATP with protein; (c) may provide direct evidence for the reversibility of the hydrolysis step. (Support by NIH grants AM17483 and GM42596 is acknowledged)

W-PM-A8 CONTRACTILE ACTIVITY OF THE PERFUSED RABBIT HEART AND THE PHOSPHORYLATION OF TROPONIN I AND MYOSIN LIGHT CHAINS. R. J. Solaro, N. Frearson*, and S. V. Perry*, Dept. of Biochemistry, University of Birmingham, Birmingham B15 2TT England.

We measured amounts of covalent phosphate attached to troponin I and myosin light chains obtained from perfused rabbit hearts, freeze-clamped at various levels of activity. Methods (affinity and ion exchange chromatography) were developed for isolation of these proteins from the same heart homogenized in 8 M urea, 75 mM Tris-HCl, pH 8.0, 1 mM $CaCl_2$. Levels of covalent phosphate during basal activity in the Langendorff procedure was 1.5 mol/mol troponin I and 1.0 mol/mol myosin light chain. In most cases changes in phosphate levels of troponin I that we (Solaro, Moir and Perry, Nature 262:615,1976) previously reported were opposite the change in myosin light chain phosphorylation. Phosphate levels of troponin I fell from 2-3 mol/mol to 1.5 mol/mol during the equilibration period of perfusion, while phosphate levels of myosin light chains rose from 0.5 to 1.0 mol/mol. Phosphate levels of troponin I rose from 1.5 to 2.5 mol/mol at the peak of the inotropic response to 2 μM epinephrine, while phosphate levels of myosin light chains fell from 1.0 to 0.5 mol/mol. Although propranolol suppressed these effects of epinephrine on both light chain and troponin I phosphorylation, propranolol alone reduced the levels of covalent phosphate of myosin light chains. Perfusion with medium containing increased Ca^{++} and reduced Na^+ had little effect on troponin I phosphorylation, but reduced myosin light chain phosphorylation. Ouabain had little, if any, effect on troponin phosphorylation, but slightly reduced the levels of phosphate of the light chains. Supported by grants from the MRC and done during the tenure by R.J.S. of a British-American Research Fellowship of the British Heart Society and the American Heart Association.

W-PM-A9 A STUDY OF MYOSIN ATPASE ACTIVATION BY F-ACTIN MONOMER. James E. Estes and Lewis C. Gershman, Dept. of Medicine, U.S. Veterans Administration Hospital, Albany, NY 12208.

At low actin concentrations (0.02-0.04 mg/ml), the rate of proteolytic digestion of monomeric actin in the presence of KCl (F-actin monomer) is similar to that of F-actin and much less than the digestibility of G-actin (Rich & Estes, J. Mol. Biol. 104, 777 (1976). The conformation of F-actin monomers thus appears to be more like that of the subunit in actin polymers than it does to G-actin monomers, and it therefore became of interest to investigate the ability of F-actin monomers to activate the ATPase of myosin.

In 0.1 M KCl the ATPase of heavy meromyosin (0.02-0.04 mg/ml) is only very slightly activated (1-1.5 fold) in the presence of F-actin monomers (actin concentrations below 0.05 mg/ml). Above 0.05 mg/ml (the critical actin concentration in 0.1 M KCl), actin activated the HMM ATPase to an extent proportional to the amount of polymer present. Fully polymerized F-actin diluted to 0.02-0.08 mg/ml activated the HMM ATPase 8-20 fold. In the presence of 0.02 M KCl, F-actin monomer is again characterized by both low digestibility and very little ability to activate the HMM ATPase, while F-actin diluted to the same conditions (0.02-0.10 mg/ml) also showed low digestibility but significant amounts of ATPase activation. Under these conditions G-actin activates the HMM ATPase 1.5-2 fold before polymerizing.

These data suggest that the conformation of F-actin monomer, while different from that of G-actin, may not be the same as the conformation of the subunits in F-actin polymer. Alternatively, significant activation of the myosin ATPase may require the polymeric state of actin.

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W-PM-A10 MAGNESIUM INHIBITION AND ACTIVATION OF ACTOMYOSIN ATPase. G. W. de Villafranca, Dept. of the Biological Scineces, Smith College, Northampton, Mass. 01060

We have previously reported the variable effects of magnesium on different preparations of Limulus (horseshoe crab) myosin B. We can now, with some consistency, prepare myosin B from myofibrils which have been washed with relaxing media (0.05 M KCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, in 5 mM phos. buffer, pH 7.5) which has good activity in 4.9 mM $CaCl_2$ and which is slightly enhanced by the addition of $MgCl_2$ to a final concentration of 3.3 mM. We designate this magnesium activation and have an average value of 106 for the magnesium-sensitivity. The traditional EGTA measure of calcium-sensitivity for these preparations is 65. The magnesium activation can be removed by washing this myosin B with a buffer at pH 7.4. The ATPase now becomes inhibited by the magnesium added to the calcium-containing medium: a magnesium-sensitivity of 60. With even greater consistency we can prepare myosin B by direct extraction of the muscle with 0.6 M KCl pH 7.9 which also shows good ATPase activity with 4.9 mM CaCl but is inhibited when $MgCl_2$ is added to the test medium: an average magnesium-sensitivity of 49. The calcium-sensitivity for these preparations is 51. When this kind of myosin B is treated with EDTA to a level of 0.1 mM the enzyme loses its magnesium inhibition and becomes strongly magnesium-activated with good activity: a magnesium-sensitivity of 127. Calcium added to the magnesium test medium not only does not activate (no calcium sensitivity) but, in fact, seems to inhibit in some cases (a negative calcium-sensitivity).

This work was supported in part by USPHS Grant AM 02647.

W-PM-A11 A THERMALLY POTENTIATED STATE FOR RABBIT SKELETAL MUSCLE ACTOMYOSIN ATPase. Anthony Giambalvo and Paul Dreizen. Department of Medicine and Program in Biophysics, State University of New York, Downstate Medical Center, Brooklyn, N.Y. 11203.

Heat treatment of natural actomyosin (NAM) isolated from bullfrog skeletal muscle results in a potentiated state for Mg ATPase (Biophysical Journal, 16, 44a, 1976). We here report that NAM isolated from rabbit skeletal muscle exhibits a similar phenomenon over a temperature range ~10°C higher than exhibited by frog NAM. Heat treatment of NAM results in substantial augmentation of Mg ATPase, moderate decrease of EDTA ATPase, and little increase, or no change in Ca ATPase. The effects of heat treatment involve a transition to a potentiated state for ATPase in that changes persist despite decrease of temperature. However, heat treated NAM does undergo reversal to the native state following incubation at low temperature under appropriate solvent conditions. Heat treatment of NAM is also accompanied by loss of Ca^{++} sensitivity, but this effect lags behind the augmentation of Mg ATPase. Moreover, the effects of heat on Ca^{++} sensitivity are irreversible. Further studies were conducted to determine the role of each of the major constituents of NAM in the potentiation process. As expected, heat treatment of actin and myosin separately is accompanied by irreversible denaturation without evidence of potentiation. Heat treatment of actin and myosin together results in augmentation of Mg ATPase of actomyosin, with the extent of potentiation most pronounced at actin to myosin ratios between 1 and 5 moles per mole. The highest levels of Mg ATPase are found following heat treatment of actomyosin in the presence of troponin and tropomyosin. The overall data indicate that thermal potentiation involves a complex interaction between actin and myosin, as modified by troponin and tropomyosin. Supported by USPHS Grants AM-06165 and HL-16596.

W-PM-A12 BINDING OF F-ACTIN TO SUBFRAGMENT 1 BEARING DIFFERENT ALKALI LIGHT CHAINS. L. Wadzinski*, S. Highsmith, A. Wang*, J. Woodard*, and J. Botts, Univ. of California at San Francisco, San Francisco, CA. 94143.

Using the procedure of Weeds and Taylor (Nature 257: 54, 1975), we purified subfragment 1 (S1) from rabbit skeletal muscle by ion exchange chromatography into 2 major peaks, S1A1 and S1A2, bearing alkali light chains A1 and A2, respectively. Polyacrylamide gel electrophoresis in the presence of SDS and urea shows clean S1A1 in the first major peak and essentially clean S1A2 in the descending part of the second. Over the pH range 6.4 to 9.8, CaATPase and CaITPase activities for both S1A1 and S1A2 are similar to those for a papain preparation of S1. With a 36-fold excess of F-actin, MgATPase activity (9 mM KCl, 1.5 mM $MgCl_2$, 0.22 mM EGTA, 1.33 mM ATP, 15 mM tris, pH 8) is slightly higher for S1A1 than for S1A2, in agreement with the results of Weeds and Taylor. Using time-resolved fluorescence depolarization with fluorescent dye-labeled S1A1 and S1A2, we measured binding of S1A1 and S1A2 to F-actin in the absence of ATP. Although binding showed a marked dependence on [KCl] over the range 0.14 - 0.43 M (with 1.5 mM $MgCl_2$, 0.5 mM EGTA, 10 mM imidazole, pH 7.0), the affinities of S1A1 and S1A2 for F-actin were not distinguishable. In 0.28 M KCl, the affinity constant for S1A1 is 3.98 ± .40 μM^{-1} (8) and for S1A2 is 4.66 ± .34 μM^{-1} (5). In 0.14 M KCl, the affinity constants are about 12-fold higher. [Ca^{++} (0.6 mM) has no effect on binding.] Addition of ATP reduces the fraction of bound S1A1 or S1A2 to a steady state level near zero. (Supported by NIH Grant Nos. HLO-6285, HLO-5251, and AM-05129, and by NSF Grant No. PCM 76-11491).

W-PM-A13 THE ASSOCIATION OF HEAVY MEROMYOSIN AND F-ACTIN. Stefan Highsmith, C.V.R.I., University of California at San Francisco, San Francisco, CA 94143.

The association of HMM and actin was studied by time-resolved fluorescence depolarization, under conditions (0.15M KCl, 0.010M Tes, 0.003M $MgCl_2$, 0.003M EGTA, pH 7, 4°C) equivalent to those previously used with S-1 (c.f. Highsmith, et al. (1976) Proc. Nat. Acad. Sci. USA, 73, 133-137). The association constant K_a was (1.6 ± 0.2) x 10^7 M^{-1}, which is about 10x greater than for S-1.

Both S-1 moieties of fully labeled HMM were completely immobilized by actin in moderate excess. Like S-1, HMM binding was greatly curtailed by increasing [KCl]. At 25°C, $\Delta H°$ = 36 ±2 kJM^{-1} and $\Delta S°$ = 260 $JM^{-1}K^{-1}$; these values are similar to those for S-1.

K_a for HMM is much smaller than would be expected if the two heads had the same intrinsic K_a as S-1 and the "second" head existed at a high effective concentration [Peller (1975) J. Supramolec. Struct. 3, 169-174]. The similar salt and temperature effects, the immobilization of both heads and small K_a indicate that the "second" head may be unable to fully bind, or is intrinsically different, or perhaps binds to the "first" head. (S.H. is a USPHS fellow. Research supported by HL-16683, NSF PCM 75-22698 and AHA 60-CI-08.)

W-PM-A14 MAGNESIUM-ADP BINDING TO ACTO-MYOSIN-S1 AND ACTO-HEAVYMEROMYOSIN. H.D. White, Dept. of Biophysics, King's College, 26 Drury Lane, London, WC2 5RLB, England.

Concentrations of MgADP greater than 0.05 mM reduce the rate of dissociation of acto-myosin-S1 and acto-heavymeromyosin (acto-HMM) measured by the disappearance of the light scattered by acto-S1 and acto-HMM in a stopped-flow fluorimeter. The data are consistent with the simple model, shown below, in which MgADP forms a ternary complex with the acto-myosins and is a competitive inhibitor for the MgATP

$$AM\cdot MgADP \overset{Kd}{\rightleftharpoons} AM \longrightarrow AM\cdot MgATP \rightarrow A + M\cdot MgATP \quad AM = \text{acto-S1 or acto-HMM}$$

binding site. The dissociation constant for MgADP is the same within experimental error for acto-S1 and acto-HMM (K_d = 0.2 ± 0.05 mM in 100 mM KCl, 5 mM $MgCl_2$, 10 mM Tris, pH 8.0, 20°C) and is fairly insensitive to temperature, pH and ionic strength. The observed rate constant for the dissociation of acto-HMM in the presence of 2.8 mM ADP at 4°C is proportional to the MgATP concentration up to a maximum of 400 sec^{-1}, where MgADP dissociation from acto-HMM becomes the rate limiting step of MgATP binding. At 20°C the rate of MgADP dissociation from acto-S1 and acto-HMM is faster than the mixing time of the stopped-flow, 1000 sec^{-1}.

The rate constant for the dissociation of MgADP from acto-HMM is at least 100 times more rapid than the maximum rate of ATP hydrolysis at comparable conditions and therefore must not be the rate limiting step of ATP hydrolysis.

W-PM-A15 NOVEL FLUORESCENCE EFFECTS FOR THE STUDY OF CONTRACTILE PROTEIN INTERACTIONS. R. Takashi*, Y. Tonomura*, and M.F. Morales, C.V.R.I., University of California at San Francisco, San Francisco, CA 94143.

Fluorescence excitation (EX) and emission (EM) peaks for SH-directed probes are respectively "IAEDANS" (340, 470nm) and 5- iodoacetamido fluorescein, "IAF" (500, 520nm). Proximity between points of probe attachments can therefore be tested by testing for energy transfer. When S-1 labeled with IAF at SH_1 associates with F-actin labeled with IAEDANS at Cys-373, strong energy transfer (system EX at 340nm shows quenched 470 EM and new 520 EM) occurs in the "rigor complex". Effect has applications to be illustrated.

According to G. Weber, a new probe, bis-1-anilino-8-naphthalene sulfonate (bANS) may have affinity for ATP-sized protein clefts. In hydrophobic environments bANS has EX, 390nm; EM, 490nm; in water, no fluorescence. bANS equilibrated with S-1 shows strong fluorescence, part (optimally 50%) of which is abolished by ATP or its relatives. Detection of bANS displacement from ATPase site has applications in kinetic characterization (to be illustrated). Trp energy transfer to bound bANS has been demonstrated as having slow relaxation phenomena following substrate binding. (R.T. is a Senior Fellow, BAHRC: Y.T. is a U.S.-Japan Exchange Professor; research supported by HL-16683, NSF PCM 75-22698, AHA 60-CI-08).

W-PM-A16 THE PRODUCTION OF FORCE BY SINGLE-HEADED MYOSIN. R. Cooke and K.E. Franks*, Department of Biochemistry and CVRI, University of California, San Francisco, CA 94143

Myosin has two heads, each of which can interact with actin and ATP. We have investigated the possibility that cooperative interactions occur between the heads by measuring the force generated by single-headed myosin in reconstituted actomyosin threads. Myosin was purified from myofibrils that had been digested for 30' with 0.004 mg/ml papain. This myosin consisted of approximately 10-20% two-headed myosin, 50-70% single-headed myosin, and 20-30% myosin rods. The amounts of these myosin species were determined from densitometer scans of polyacrylamide gels run in 20 mM PP_i. The myosin was combined with actin, threads were formed via extrusion, and the isometric forces and isotonic velocities generated by the threads were measured. Extensive work has shown that the tension generated by two-headed myosin is linearly proportional to its concentration in the threads (Crooks, R. and Cooke, R., J. Gen. Physiol., in press). The presence of myosin rod did not affect the tension generated by actomyosin threads. Thus the tension generated by the two-headed myosin could be calculated and subtracted from the total tension to give the tension generated by the one-headed myosin. The ratio of the isometric tension produced per head by the one-headed myosin to the isometric tension produced per head by the two-headed myosin was 1.1 $\pm$ 0.6 (range of 0.4 to 2.0, average of 6 preparations). The maximum velocity of thread contraction for the one- and two-headed myosin mixtures was not significantly different from the control two-headed myosin. Thus, the absence of one head does not appear to seriously impair the generation of force or motion by the remaining head. (This work was supported by USPHS AM 17559 and NSF BMS 14793; RC was an Established Investigator of the AHA.)

W-PM-B1 CHANGES IN ELECTRICAL PROPERTIES OF THE CELL MEMBRANE DURING "DIFFERENTIATION WITHOUT CLEAVAGE" IN THE EGG OF THE ANNELID, CHAETOPTERUS PERGAMENTACEUS. S. Hagiwara and S. Miyazaki*, Department of Physiology, U.C.L.A. Los Angeles, California 90024.

After a short exposure to high K^+ sea water (100 mM K^+, about 1 hour) unfertilized eggs of the annelid differentiate without cleavage (Lillie, Arch. F. Entwicklung. d. Organ. 14: 417, 1902), becoming ciliated in 12 - 15 hours at 22° C. Ciliated cells are analogous to trochophore larvae found during the normal development. This suggests that the differentiation of the 'cell membrane' under this condition is analogous to that occurring during the normal development. Since the size of the cell remains large (about 100 μm) throughout the stages, the preparation offers an opportunity to study changes in the membrane electrical properties during differentiation. The resting potential of the cell immersed in the artificial sea water ranges between -65 and -75 mV at all stages. The membrane of the untreated unfertilized cell is characterized by two features. (1) The cell membrane produces an action potential which depends exclusively on the external Ca^{++}. (2) The outward rectification due to the increase of the K conductance is absent at least for the range of the membrane potential less negative than +20 mV. After 12 - 15 hours cells are ciliated. In this stage, (1) a clear outward rectification appears when the membrane potential is made more positive than -20 mV and (2) the action potential depends not only on the external Ca ions but also on the external Na ions. (Supported by USPHS Grant NS 09012)

W-PM-B2 CALCIUM TRANSPORT IN INTERNALLY-DIALYZED SQUID AXONS: EFFECTS OF INTERNAL AND EXTERNAL CATIONS AND ATP. M.P. Blaustein, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

Calcium-45 efflux was measured in squid axons whose internal solute concentration was controlled by means of internal dialysis. Most of the Ca efflux requires either external Na (=Na-Ca exchange) or external Ca plus an alkali metal ion (=Ca-Ca exchange; cf. Blaustein & Russell, J.Membrane Biol. 22:285,1975). Both Na-Ca and Ca-Ca exchange are apparently mediated by a single mechanism because both are inhibited by Sr and Mn, and because addition of Na to an external medium optimal for Ca-Ca exchange inhibits Ca efflux (="occlusion"). The transport involves simultaneous (as opposed to sequential) ion counterflow because the fractional saturation by internal Ca (Ca_i) does not affect the external Na (Na_o) activation kinetics; also, Na_o promotes Ca efflux whether or not an alkali metal ion is present inside, whereas Ca-Ca exchange requires alkali metal ions both internally and externally (i.e., internal and external sites must be appropriately loaded simultaneously). ATP increases the affinity of the transport mechanism for both Ca_i and Na_o, but it does not affect the maximal transport rate at saturating [Ca_i] and [Na_o]; this suggests that ATP may be acting as a catalyst and not as an energy source. Hill plots of the Na_o activation data yield slopes ≈ 3 for both ATP-depleted and ATP-fueled axons - compatible with a 3 Na^+-for-1 Ca^{2+} exchange. Na influx and Ca efflux, measured under comparable conditions, also indicate a 3-for-1 stoichiometry (Blaustein & Russell, loc. cit.). With this stoichiometry, the Na electrochemical gradient, alone could provide sufficient energy to maintain ionized [Ca_i^{2+}] in the physiological range (about 10^{-7}M). (Supported by NSF).

W-PM-B3 BUFFERING CAPACITY OF SQUID AXOPLASM. F. J. Brinley, Jr., J. Teresa Tiffert, and A. Scarpa, Department of Physiology, University of Maryland, School of Medicine, Baltimore, Md. 21201 and Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104.

Changes in ionized calcium were studied in axons isolated from living squid by measuring absorbance of the Ca binding dye Arsenazo III using multiwavelength differential absorption spectroscopy. Absorption changes measured in situ were calibrated in vitro using media of ionic composition similar to axoplasm containing CaEGTA buffers. Axons were cleaned and stored in 3 mM saline to avoid the calcium loading of axons known to occur in 10 mM Ca seawater. (DiPolo et al, J. Gen. Physiol. 1976, 67, 433-467). Calcium loads of 50-2500 μmole/kg axoplasm were induced by microinjection, by stimulation in 112 mM Ca seawater, or by soaking in choline saline with 1-10 mM Ca. Over this range of calcium loading of intact axoplasm, the ionized calcium in the axoplasm rose about 1 nM/μM load, i.e. 99.9% of the calcium load was buffered. Similar loading in axons pretreated with FCCP (carbonyl cyanide 4-trifluoromethoxyphenylhydrazone) to inhibit the mitochondrial proton gradient incremented the ionized calcium by 5-7% of the imposed load, i.e. 93-95% of the calcium load was buffered by a process insensitive to FCCP. This FCCP insensitive buffer system was not saturated by the largest calcium loads imposed, indicating a capacity of at least several mM. Treatment of previously loaded axons with FCCP or apyrase plus cyanide produced rises in ionized calcium which could be correlated with the extent of the load. Analysis of results indicated that whereas only 5% of the endogenous calcium in fresh axons is stored in the FCCP sensitive (presumably mitochondrial) buffer system, about 30% of an imposed exogenous load in the range 50-2500 μM is taken up by this system. Support by NIH grants: NS-08336, HL-18708, and 7F22NS00021-03.

W-PM-B4 INCREASE OF THE Ca COMPONENT OF ACTION POTENTIALS DURING REPETITIVE FIRING OF APLYSIA GIANT NEURON. R. Horn* & J.J. Miller* (Intr. by D. Junge) Department of Anatomy, University of California, Los Angeles 90024.

Increase in the Ca influx during successive spikes has been implicated in the process of synaptic facilitation. The TEA (tetraethylammonium) plateau in the neuron R2 of Aplysia is maintained by Ca influx, as shown by (1) aequorin injection (Stinnakre & Tauc, 1973), (2) variation in external Ca concentration (Bryant, 1976), & (3) effect of Ca blocking agents, cobalt & cadmium (Horn & Miller, 1976). In 25 mM TEA a somatic spike has a 30-40 ms plateau. The duration of the distal axon spike does not increase in 25 TEA at frequencies <2/s. The R2 soma, but not axon, can fire an all-or-none Ca spike in Na free medium (Junge & Miller, 1974). The above facts suggest that the duration of the TEA plateau represents the degree of Ca activation during a spike. The duration of the TEA plateau increases during repetitive firing (RF), indicating an increase in Ca influx for successive spikes. The time course of the facilitation of the TEA plateau is comparable with that of neuromuscular facilitation in Aplysia (Orkand & Orkand, 1975). This increase in plateau duration during RF is found in both the soma and axon of R2, and is observed for either intra-or extracellular application of TEA. The broadening action of TEA during RF is blocked by 30 mM Co^{++}, or Ca free solutions. In the absence of TEA, studies of the ionic dependence of the overshoot potential and the rate of rise of spikes show that successive spikes in a train are more Ca dependent and less Na dependent.

W-PM-B5 CALCIUM AND CALCIUM ACTIVATED ION FLUX IN ISOLATED SOMATA OF MOLLUSCAN NEURONS. J. A. Connor, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

A voltage-dependent transmembrane calcium flux has been recognized for some time in the neural somata of gastropod mollusks [Geduldig and Jung (1968), J. Physiol. 199, 347]. More recently it has been demonstrated that elevated internal calcium produces an increase in potassium conductance [Meech (1972), Comp. Biochem. Physiol. 42A, 493]. The time course of the calcium flux and the nature of the potassium activating mechanism are uncertain. Studies have been carried out on isolated somata from Archidoris montereyensis in which all currents but these two were minimized by external tetraethylammonium ion and appropriate conditioning voltage. The calcium flux is strongly activated for membrane voltage >0 mV and deactivates rapidly ($\tau \sim 2$ msec at 8°) near the resting potential. Following a voltage step, net current is first inward then outward, but the change in direction is apparently due to I_K activation, not I_{Ca} inactivation. Following injection of EGTA, which presumably holds internal calcium at a low concentration, voltage steps produce inward current which is maintained for several seconds with little inactivation. Equimolar substitution of Ba or Sr for external Ca gives rise to the same steady inward current pattern without EGTA injection. Magnitude of the inward current carried by Ba or Sr is comparable to the peak magnitude in Ca, indicating that the membrane is not strongly selective against either. These substitutes apparently do not block the Ca-activated I_K, but merely fail to activate it. Supported by NSF GB 39946.

W-PM-B6 UNIFIED THEORY OF 1/f AND CONDUCTANCE NOISE IN NERVE MEMBRANE. J.R. Clay and M.F. Shlesinger*(Intr.by R.L. DeHaan), Anatomy Dept.,Emory Univ.,Atlanta, GA 30322 and Physics Dept., Univ. of Rochester, Rochester NY 14627.

The power spectrum of membrane current noise from depolarized nerve axons appears to be a simple sum of 1/f and conductance terms($10\ \text{Hz} \lesssim f \lesssim 10^3\text{Hz}$). Conti,*et al.*(J.Physiol.248:45, 1975)have shown that 70 mM TEA suppresses K conductance noise, as well as partially suppressing 1/f noise. This finding,together with the voltage dependence of the current noise, suggests that the single K channel spectrum is *also* a simple sum of 1/f and conductance terms. Theoretically, the single channel spectrum should contain a third term representing the interaction of the two noise types. We have examined this point by extending our model of 1/f noise(Clay & Shlesinger,Biophys.J.16:121,1976) to channels having HH gating molecules following the approach of Hill and Chen(Biophys.J.12:948,1972). Our basic model is a mathematically and physically self-consistent description of one possible way in which an ionic channel may interact with the fluid motion of a lipid bilayer. The model requies that the channel-channel distance be much larger than lipid-lipid distance. Consequently, the random motion (primarily rotational)of any one channel is stochastically independent of the other channels. Including noise from channel gates, we find that the total noise is a sum of three terms: 1/f and conductance noise which both increase linearly with number of channels N,and a term given by the product of single channel 1/f and conductance terms which is *independent* of N. Therefore, the spectrum is effectively a sum of the dominant noise types. Since ionic current in our model is produced by non-energy dissipating collisons of external ions with channel ions, we also predict 1/f noise in the absence of gradients which is consistent with the data of Poussart(Biophys.J. 11:211,1971). Equilibrium 1/f noise has also been observed recently in small metal films by Voss and Clarke(Phys.Rev.Lett.36:42,1976).

W-PM-B7 ONSET OF REPETITIVE ACTIVITY FOR A POINT-STIMULATED THEORETICAL AXON. J. Rinzel*, (Intr. by W. Rall), Mathematical Research Branch, NIAMDD, National Institutes of Health, Bethesda, MD. 20014.

In response to point stimulation with a current step, a neuron may propagate a repetitive train of action potentials along its axon. For steady repetitive activity, the current strength I must typically lie in an appropriate range $I_1 < I < I_2$. For I outside this range, time independent steady state behavior is usually achieved after an initial transient. If I is slowly varied through the critical values of I_1 and I_2 the steady frequency ω is observed to jump discontinuously between zero and some finite value. Analytical results for an analogous theoretical cable problem agree with this qualitative experimental description. For this we consider a two-variable (FitzHugh type) nerve conduction equation with an instantaneous N-shaped current-voltage law. For each value of the steady point source current intensity I, the equation has a time-independent, steady state solution (decrement with distance). At first, this may seem inconsistent with the experimental observations. To reconcile it we use mathematical perturbation theory to study the stability of the steady state solution. For small or large I it is a stable solution; this corresponds to experiments with $I < I_1$ (sub-threshold) and $I > I_2$ (beyond high frequency cut-off). For an intermediate range of I values, the steady state solution is unstable; it corresponds to a potential distribution that would not be observed experimentally. This intermediate range thus lies interior to the range of I values for repetitive activity. For two critical values of I (at which the steady state loses or gains theoretical stability) our analysis indicates that a branch of periodic solutions, with non-zero frequency, arises. This is consistent with the observed discontinuities in the experimental ω-I curve.

W-PM-B8 MEMBRANE CONDUCTANCE AND PERMEABILITY OF Na, K, AND Cl IN NERVE AS A FUNCTION OF MEMBRANE POTENTIAL DURING ZERO MEMBRANE CURRENT. A. Strickholm, Physiology Section, Medical Sciences Division, Indiana University, Bloomington, Indiana 47401

Medial giant axons from crayfish (Procambarus clarkii) were isolated in crayfish saline at pH 7.0. Specific membrane conductance G_m was determined from the axon cable properties by inserting axially from opposite ends a current injecting and a voltage recording electrode which provided the length constant and cable input impedance. The ionic dependency of the membrane potential on a jth ion (transference number T_j) was obtained by a step change in the jth external ion concentration and recording the corresponding change in membrane potential ΔV_m. From G_m, T_j, and V_m, specific ionic conductances and permeabilities were calculated from classical electrodiffusion theory. These measurements (G_m, T_j, and V_m) were made continuously for K, Na, and Cl, while the membrane potential normally dropped to zero over many hours while maintaining near zero membrane current. The results showed essentially no change in membrane conductance until around -30 mV membrane potential, below which total conductance rapidly begins to increase. From -90 to -30 mV, g_K and g_{Na} slightly decrease during membrane depolarization while g_{Cl} slightly increases. The calculated ionic permeabilities similarly show minimal change over this potential range. One interpretation of this data is that potassium activation, which occurs with depolarization in a normal axon membrane, requires a non-zero membrane current which alters local surface membrane charges, since depolarization alone at zero membrane current produces no potassium activation. This experimant supports the view that changes in local ion concentrations at the cell surface membrane, which have been shown to produce a membrane protein conformation change (Clark and Strickholm, Nature, 234: 470) are involved in the action potential process.

W-PM-B9 A PHYSICAL MODEL OF NERVE AXON: ACTION POTENTIAL AND VOLTAGE CLAMP CURRENTS. D. C. Chang, Physics Department, Rice University, Houston, TX 77001, and Baylor College of Medicine, Houston, TX 77030

In the last Biophysical Society Meeting I showed that one can calculate the ionic distribution and surface charge at the axon surface using a model which is based on a set of assumptions different from those of the ionic theory of Hodgkin et al. The mobile ions are considered to be distributed at Donnan equilibrium, and the axoplasm is regarded as an analog of a cation exchanger. This model can describe the relationship between the resting potential and the ionic concentrations very close to what was experimentally observed. I have extended this model to explain the action potential and the ionic currents under voltage-clamp conditions. Unlike the ionic theory which attributes the Na^+ inward current solely to a change of sodium permeability, I propose that attached to the membrane there is a layer of axoplasm which can undergo conformational changes and hence can modulate the selectivity for mobile ions. The selectivity is controlled by a cooperative mechanism. A change of ion selectivity of this axoplasm layer will result in an onset of the transient currents. Detailed theoretical analysis and data from voltage-clamp experiments in perfused squid giant axon will be presented.

W-PM-B10 A KINETIC MODEL FOR SLOW INACTIVATION IN NERVES.

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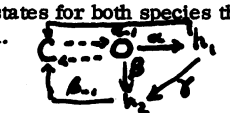
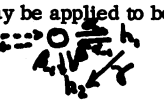
During a short depolarization (less than 10 msec.) 15% of the sodium channels in Myxicola giant axons enter an inactivated state (h_2) from which recovery is very slow. A slow inactivated state is reached by most of the sodium channels if the membrane is depolarized for several seconds. It seems reasonable to identify this state with the h_2 state because the recovery time constants measured at various potentials correspond well. (Schauf, Biophys. J. 16: 771, 1976). In contrast, in squid giant axons it appears as if the h_2 state is only reached by long depolarizations. However, if the inactivated state which reactivates quickly (h_1 = h of Hodgkin and Huxley) is blocked by internal perfusion with proteolytic enzymes, the h_2 state persists but develops 2.5 times faster. Chandler and Meves obtained the same result for the fraction of the sodium currents which lack h_1 in NaF perfused axons. While the results can be interpreted by models which propose different "slow inactivated" states for both species the schemes below may be applied to both and are consistent with the data.

C: closed
O: open



OR



$\alpha, \alpha_1, \beta, \gamma$ (SIMILAR FOR SQUID AND MYXICOLA)

$\alpha \gg \gamma$; $\alpha_1 \gg \beta$ In Myxicola and squid.

$\beta \gg \gamma$, $\frac{\beta}{\alpha+\beta} \cong .15$ (In Myxicola) $\beta \approx 25\gamma$, $\frac{\beta}{\alpha+\beta} \cong 1 \times 10^{-3}$ (In Squid)

I acknowledge Dr. L. Goldman's suggestions regarding the model.

W-PM-B11 COOPERATIVE ACTIVATION AND IONIC DEPENDENCE OF NEUROTOXIN-STIMULATED ^{86}Rb EFFLUX FROM NEUROBLASTOMA CELLS. C. Palfrey*, Department of Pharmacology, Yale University School of Medicine, New Haven, Ct. 06510 (Intr. by D. Campbell)

The neurotoxins veratridine (V), batrachotoxin (BTX) and scorpion venom (Leiurus quinquestriatus; SV) all activate the voltage-dependent Na^+ channels of excitable membranes, and lead to stimulation of ^{22}Na influx in cultured neuroblastoma cells (Catterall, PNAS, 72, 1782 (1975)). We have previously shown that V also stimulates ^{42}K and ^{86}Rb efflux from the same cells in a TTX-sensitive manner, and proposed that this effect was mediated by the Na^+ channel itself (Palfrey and Littauer, BBRC, 72, 209 (1976)). Further studies employing BTX and SV showed that both agents could replace V in the induction of ^{86}Rb efflux. BTX was maximally and immediately effective at 0.5 μM, but at lower concentrations a well-defined lag period was present. SV (5 μg/ml) was only slightly effective alone, but when combined with V (20-100 μM) led to a synergistic activation of efflux, greater than the sum of the individual effects of the toxins. These results are analogous to those obtained with ^{22}Na influx (Catterall, loc. cit.), and suggest a heterotropic cooperative interaction between nonidentical sites for alkaloids and SV associated with the Na^+ channel. Stimulation of ^{86}Rb efflux by V or BTX alone was totally dependent on the presence of extracellular Na^+, replacement of the latter with choline$^+$, Tris$^+$, Rb^+ or Cs^+ abolishing activation. The relative ability of other monovalent ions to support alkaloid-mediated efflux was $Na^+ > Li^+ > K^+$. In contrast, incubation in the presence of toxin combinations (V + SV, or BTX + SV) eliminated the extracellular Na^+ requirement, and activation was obtained in the presence of all monovalent ions tested. These experiments rule out the possibility that the extra ^{86}Rb efflux might be secondarily related to Na^+ influx or depolarization, and suggest that SV may be binding at a regulatory site on the Na^+ channel normally occupied by Na^+ itself.

W-PM-B12 EVIDENCE FOR IONIC INTERACTIONS IN THE K CHANNEL OF SQUID GIANT AXONS. T. Begenisich, and P. DeWeer, Department of Physiology, University of Rochester, and Department of Physiology, Washington University.

We report here the results of our efforts to determine whether or not K ions pass through the K channels of squid axon membrane independent of one another. The experiments were done with voltage-clamped internally perfused squid giant axons. Sodium channels were blocked with 300 nM tetrodotoxin. We measured the K^{42} efflux at 3 different membrane depolarizations (20,30 and 40 mV) from a holding potential of -78 mV. At each membrane voltage the K^{42} efflux was dramatically reduced when the external K concentration was raised. For example, at a membrane potential of -48 mV the efflux was reduced by 75% by a change of external K from 10 to 75 mM. This efflux was blocked by external application of 2 mM 4-aminopyridine, which is known to block the current through the K channels of this preparation. These results show that K ions do not move independently through the K-channel but interact with each other as they cross the membrane. (Supported by N.I.H. Grant 5P01 NS10981).

W-PM-B13 INTERACTIONS BETWEEN INTERNAL SODIUM IONS AND THE POTASSIUM CHANNEL OF THE SQUID GIANT AXON. R.J. French*, J.B. Wells, Laboratory of Biophysics, NINCDS, Marine Biological Laboratory, Woods Hole, Mass. 02543

Voltage clamp experiments were performed on internally perfused squid giant axons bathed in TTX-ASW. With K as the only internal cation, the "instantaneous" I-V curve was linear at positive voltages up to +240 mV(currents were measured 100 μsec after a 10 msec prepulse to +60 mV). When Na was added to the internal solutions at concentrations of 25 mM or greater with $[K]_i$ = 300 mM, an N-shaped instantaneous I-V characteristic was seen. At moderately positive voltages a region of negative slope was seen, apparently due to the blocking of the K channels by Na ions (see Bezanilla and Armstrong, J. Gen. Physiol. 60: 588-608, 1972). At higher voltages (≳ + 160 mV) the currents increased at all sodium concentrations used (10-200 mM). Normal linear I-V relations were seen on return to control solutions. Throughout the whole voltage range, currents could be blocked by the addition of internal tetraethyl ammonium (TEA) ions.

The following experiments suggest that the observed relief of the Na block at high voltages was due to the forcing of Na ions through the K channel: (a) With Na as the only cation in the internal perfusion fluid, the I-V curve turned sharply upward at about + 160 mV, (b) A large fraction of this current was blocked by internal TEA, and (c) With K-free internal solutions, the outward currents were larger for higher internal Na concentrations.

W-PM-B14 A MODEL FOR Cs BLOCKING OF THE K CONDUCTANCE IN ANOMALOUS RECTIFICATION. S. Ciani, S. Hagiwara and S. Miyazaki*, Dept. of Physiol. and Brain Res. Inst., U.C.L.A., Medical School, Calif. 90024.

Hagiwara et al. (J. Gen. Physiol. 67, 621, 1976) have shown that Cs blocks the inward K flux in starfish egg cell membranes according to the equation, 1/R=1+B[Cs]. R is the ratio of the K conductance in the presence of Cs to that in the absence of Cs; B depends on [K] and increases exponentially with negative membrane potentials ($B \propto \exp(-\mu\phi)$ with μ=1.5. Note that since $\mu > 1$, μ cannot be interpreted as the fraction of membrane potential at the blocking site). All these features are accounted for by a model based on the following assumptions: 1) The permeation path is a two-site pore; 2) Cs blocks the pore by binding to the inner site; 3) the presence of doubly occupied, non-conducting pores, with 1 Cs strongly bound to the inner site and 1 K to the outer one, is allowed for. The expression for the conductance ratio, R, deduced from the model is,

$$1/R = 1+B^{o}_{Cs}[Cs]\exp(-\phi_2)+B^{o}_{Cs}B^{Cs}_{K}[Cs][K]\exp(-\phi_2-\phi_1).$$

B^{o}_{Cs} is the binding constant for Cs to the inner site in an empty pore and B^{Cs}_{K} is the binding constant for K to the outer site in a pore already occupied by Cs. ϕ_1 and ϕ_2 are the potentials at the outer and inner site, referred to that of the external medium. For [Cs] =5×10^{-4} M and [K]=10^{-2} M, 2.5×10^{-2} M and 5×10^{-2} M, a remarkably good fit of the data shown in the paper referred to above is obtained using the values, $B^{o}_{Cs}B^{Cs}_{K}$=140 M^{-2}, $\phi_1+\phi_2=\mu\phi$ with μ=1,6, and assuming that the second term on the right hand side of the equation is negligible. (Note that a value greater than 1 for μ is compatible with the model, since $\phi_1+\phi_2$ may be larger than the membrane potential, ϕ.)

(Supported by USPHS Grant NS 09931 and USPHS Grant 09012).

W-PM-B15 BLOCKING SITES IN K PORES. C.M. Armstrong and F. Bezanilla., Dept. of Physiol., Univ. of Pennsylvania, Philadelphia, PA 19174.

A K pore in squid axon membrane is thought to have two sites occupiable by blocking ions, one inside and one outside. The inner site can be occupied, when the gate of the pore is open, by blocking ions eg. TEA^+, Cs^+, and 3 phenylprophyltriethylammonium ion (ϕC_3). The outer site can be occupied by Cs^+. We have performed three experiments to get a clearer idea about these sites. Expt. 1: In an axon perfused with 450 mM K^+ and .5 mM ϕC_3, recovery from block by ϕC_3 was studied. 100 mM K_o speeds recovery by about 4 times (relative to K free sea water). 100 mM Cs_o has no effect. 100 mM K_o + 75 mM Cs_o speeds recovery by 1.8x. Conclusions are: a) Cs does not penetrate well enough to clear ϕC_3 from the inner blocking site, b) K does, c) Cs can occupy the outer site while ϕC_3 is in the inner. This inhibits pore clearing by K_o. Expt. 2: Entry of internal ϕC_3 into the inner site is 4x slower when K^+ is replaced by tetramethylammonium (TMA) as the main internal cation. Conclusion: the blocking reaction is

$$\phi C_3 + \text{K-site}_i \rightleftharpoons \phi C_3\text{-site}_i + K_o$$

TMA cannot pass through the pore; an analogous reaction cannot occur; and ϕC_3 thus can occupy only empty sites, which are rare. Expt. 3: Cs inside an axon slows the closing of K channel gates. This is consistent with the reaction closed↔open↔Cs-blocked, which implies that the pore gate cannot close with Cs^+ in the inner site.

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W-PM-C1 NEUTRON DIFFRACTION OF CHOLESTERYL MYRISTATE IN DIFFERENT PHYSICAL STATES. B.E North, G.G. Shipley, D.M. Small, D.M. Engelman, and B.P. Schoenborn, Biophysics Division, Boston University School of Medicine, Boston, Mass. 02118, Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, Conn. 06520, and Biology Dept., Brookhaven National Laboratory, Upton, N.Y. 11973.

In human serum low density lipoproteins and in atherosclerotic lesions, cholesterol esters exist in smectic-like liquid crystalline phases. Although the optical properties of smectic mesophases suggest a layered structure in which the long axis of the molecules is aligned perpendicular to the layers, the detailed structure of the smectic phase of cholesterol esters is not well understood. Using neutron diffraction, we have examined cholesteryl myristate and cholesteryl perdeuteromyristate in the crystalline phase, smectic and cholesteric mesophases, and in the isotropic phase. In the smectic phase, the neutron diffraction of cholesteryl myristate (and cholesteryl perdeuteromyristate) consists of a single sharp maximum at $s = 0.030\ A^{-1}$, related to molecular layering, and diffuse scattering at $s = 0.2\ A^{-1}$, related to the side-by-side packing. The sharp maximum broadens in the cholesteric phase and disappears in the isotropic phase. In addition, the deuterated compound exhibits a broad scattering also centered at approximately $0.03\ A^{-1}$ which persists, although at slightly higher scattering angles, in the cholesteric and isotropic phases. Differences in the crystal diffraction patterns for the hydrogen and partially deuterated compounds are readily explained on the basis of the known crystal structure of cholesteryl myristate (B. Craven & G. DeTitta, J. Chem. Soc., Perkin II (1976) 814). We are currently investigating structural models which can account for the diffraction from the smectic phase.

W-PM-C2 STERIC EFFECTS ON THE CONFORMATION OF HYDROCARBON CHAINS AS STUDIED BY NEUTRON SCATTERING. F. Goodsaid*, D.M. Engelman, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Conn. 06520

The role of steric forces in determining the conformation of the hydrocarbon chains of lipid bilayers and membranes is not well documented. One approach is to study the conformation of isolated hydrocarbon chains of moderate length in different physical environments, including the liquid phase of pure chains. To measure the conformation of a single chain in an environment of other identical chains, deuteration is used in a small-angle neutron scattering experiment. Measurements of the molecular radius of gyration can be made with different environments, ranging from solvents which do not restrict the conformation to the high levels of constraint imposed by the solid state of the pure compound. Studies of a series of normal alkane chains including n-dodecane (C12), n-hexadecane (C16), and n-eicosane (C20) will be reported. These studies show that "staining" of a molecule with deuterium permits neutron diffraction studies of its conformation in the liquid state. These studies should extend to the study of lipid species in membranes, as well as the study of other pure phases.

(This work was supported by USPHS-HL-14111.)

W-PM-C3 NEUTRON DIFFRACTION STUDIES OF THE STRUCTURE OF ORIENTED DIPALMITOYL LECITHIN MULTILAYERS. A.M. Saxena* and B.P. Schoenborn, Biology Department, Brookhaven National Laboratory, Upton, New York 11973.

Structure of DPL multilayers has been analyzed by Zaccai, Blasie, Schoenborn (1975)† and Worcester and Franks (1976)‡. The resolution in these experiments was ~ 6Å, which was essentially determined by the mosaic spread of the multilayer. In the present experiments the resolution has been increased to ~ 4Å by making well oriented multilayers. It is also found that the structure factors of various orders of reflections should be corrected for a number of geometrical factors. These corrections arise due to the usual Lorentz factor, vertical divergence of the slits, and the change in the volume of the sample exposed to the incident beam with the order of reflection.

Samples were prepared by placing a suspension of DPL in water on a quartz slide, and allowing it to dry in a 15% humidity atmosphere. The multilayer was then annealed by heating it to about 110°C in a nitrogen atmosphere and then cooling it at a very slow rate. This produced DPL multilayers with a mosaic spread of 0.7°.

Integrated intensities of various orders were measured by taking rocking curves of the sample with a position sensitive detector. Phases were assigned to structure factors of different orders by H_2O - D_2O exchange using the conventional Patterson function technique. Since the mosaic width was small, 14 reflections were recorded which led to structure determination with a resolution of ~ 4Å. Research carried out under the auspices of the U. S. Energy Research and Development Administration.

† Zaccai, G., Blasie, J. K., and Schoenborn, B. P. (1975). Proc. Nat. Acad. Sci. USA 72, 376.

‡ Worcester, D. L., and Franks, N. P. (1976). J. Mol. Biol. 100, 359.

W-PM-C4 THE X-RAY INTERCHAIN PEAK PROFILE IN DIPALMITOYLGLYCEROPHOSPHOCHOLINE (DPPC)
G. W. Brady and D. P. Fein*; N. Y. State Dept. of Health, Albany, N. Y. 12208

The primary x-ray peak profile characterizing the interchain structure in the DPPC membrane has been measured as a function of temperature. The scattering between 23°C and 34.6°C is characterized by an asymmetric crystalline reflection accounting for 85% of the total intensity, the remaining 15% being liquid-like in character. AT a pre-transition temperature of 34.6°C, the reflection profile becomes (nearly) symmetrical, indicating a change in tilt angle of the chains with respect to the membrane surface. This change is accompanied by an increase of 20% in the amount of liquid-like scattering, indicating that the pre-transition mechanism includes a partial melting of the chains. At the melting point, 41.5°C, the crystalline reflection disappears, and the liquid component of the scattering increases to a point where it includes all the scattered intensity. The relative values of the integrated intensities at each temperature are tabulated, and the significance of the peak widths and shapes are discussed.

W-PM-C5 LOW AND HIGH ANGLE X-RAY DIFFRACTION OF ORIENTED L-DIPALMITOYL PHOSPHATIDYLCHOLINE (DPPC) MULTILAYERS.
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Diffraction data over a large angular region have been gathered on highly oriented multilayers of DPPC using a precise three-crystal diffractometer and a 50 KW rotating anode x-ray source. Both monohydrate (2% H_2O content) and samples containing 10% water (both exhibiting optical biaxiality at room temperature) have been studied. The distribution of intensity at the 4.2Å reflection has been examined and found to be dependent upon water concentration. Two possible theoretical models will be discussed to explain these data:

a) Terraced crystal structure normal to the bilayers;

b) Tilting of the hydrocarbon chains.

In addition, lamellar reflections up to 21 orders have been recorded for samples with these two water contents.

W-PM-C6 DILATOMETRY OF LIPID BILAYERS.
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A differential dilatometer has been constructed for measuring temperature induced volume changes in lipids. The device is of closed system design, the expansion of the sample and reference liquids being taken up by metal bellows. The resulting linear movement of the bellows is measured using a Fizeau interferometer with a helium-neon laser source. The combination of high sensitivity (20 nanoliters) with the differential aspect of the instrument allows the use of small amounts (50 to 100 mg) of lipid at low concentration (0.5% to 1%) in water. Two other aspects of this device are important in regard to lipids. The sample can be continuously stirred to prevent settling out of the lipid dispersions and true equilibrium measurements can be made even when equilibration times are several hours. The phase transitions of dipalmitoyl phosphatidycholine liposomes have been studied. In agreement with our earlier study[1] and one independent study[2], we obtain a 3.5% change in volume for the main transition at 41°C. For the lower transition at 35°C a 0.35% change is obtained and this transition displays considerable hysteresis. The coefficients of expansion of the three phases studied (below lower transition, between the two transitions, and above the main transition) have also been measured and have been found to be different from each other.

1. J. F. Nagle, Proc. Nat. Acad. Sci. USA 70, 3443 (1973).
2. J. F. Blazyk, D. L. Melchior and J. M. Steim, Anal. Biochem. 68, 586 (1975).

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W-PM-C7 ENERGETICS OF LIPID BILAYER PHASE TRANSITIONS. J.F. Nagle, Departments of Physics and Biological Sciences, Carnegie-Mellon University, Pittsburgh, Pa. 15213

The precision volumetric measurements discussed in the preceding paper are used in conjunction with calorimetric data to discuss the energetics of the gel-liquid crystal phase transition in lipid bilayers. The two largest increases in energy as T is raised through the transition are (1) $\epsilon \Delta n_g$ where ϵ is the trans-gauche isomerization energy and (2)the increase in van der Waals hydrocarbon interchain energy. It will be shown how the latter energy can be estimated from the volume change. The relatively small magnitude of the other energy changes will be discussed. From the experimental data and the assumption that n_g is nearly zero in the gel phase we estimate that n_g is about 7 or 8 per dipalmitoyl phosphatidycholine molecule in the liquid crystal phase, which may be compared to $n_g = 12$ for a hypothetical perfectly isotropic, effectively noninteracting phase. It is concluded that the gel-liquid crystal phase transition is primarily due to the competition between excluded volume interactions, trans-gauche isomerizations and isotropic van der Waals attractive dispersion interactions. The primary effect of the water is to pin the headgroups to a planar surface, thereby providing a unique axis. Headgroup interactions are secondary in importance, although they are biologically very significant and have been incorporated into ab initio statistical mechanical calculations.[1]

1. J. F. Nagle, J. Membrane Biol. 27,233 (1976).

Research supported by NSF grant DMR 72-03203-A02.

W-PM-C8 EFFECTS OF TEMPERATURE AND MOLECULAR INTERACTIONS ON THE VIBRATIONAL INFRARED SPECTRA OF PHOSPHOLIPID VESICLES. I.M. Asher, Food and Drug Administration, Rockville, Maryland 20852, and I.W. Levin,* Laboratory of Chemical Physics, National Institutes of Health, Bethesda, Maryland 20014.

Infrared spectra were obtained as a function of temperature for a variety of phospholipid/water bilayer assemblies (80% water by weight) in the 3000-950 cm^{-1} region. Spectral band-maximum frequency parameters were defined for the 2900 cm^{-1} hydrocarbon chain methylene symmetric and asymmetric stretching vibrations. Temperature shifts for these band-maximum frequencies provided convenient probes for monitoring the phase transition behavior of both multilamellar liposomes and small diameter single-shell vesicles of dipalmitoyl phosphatidylcholine/water dispersions. As examples of the effects of bilayer components upon phase transition characteristics, the temperature profiles for lipid/cholesterol/water (3:1 mol ratio) and lipid/cholesterol/amphotericin B/water (3:1:0.1 mol ratios) vesicles were examined using the methylene stretching frequency indices. In comparison to the pure vesicle form, the transition width of the lipid/cholesterol system increased by nearly a factor of two (to 7°C) while the phase transition temperature remained approximately the same (41°C). For the lipid/cholesterol/amphotericin B system, the phase transition temperature increased by about 4.5°C (to 45.5°C) with the transition width increasing by nearly a factor of four (to ∿15°C) above that of the pure vesicles. The lipid/cholesterol/amphotericin B data were interpreted as reflecting the formation below 38°C of a cholesterol/amphotericin B complex whose dissociation at higher temperatures (38-60°C range) significantly broadens the gel-liquid crystalline phase transition.

W-PM-C9 THERMODYNAMICS AND STRUCTURES OF MIXED PHOSPHOLIPIDS: LYSOMYRISTOYLPHOSPHATIDYLCHOLINE (LMPC) AND DIMYRISTOYLPHOSPHATIDYLCHOLINE (DMPC). F.-J. Hsu,* A. M. Gotto, Jr.* and H. J. Pownall. Division of Atherosclerosis and Lipoprotein Research, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030.

The enthalpies of association of LMPC micelles (CMC = 45 μM) and sonicated DMPC vesicles were recorded as a function of mole fraction of reactants and temperature. At an LMPC/DMPC molar ratio of 1:2, the maximum enthalpy of association was at 23.7°, the gel → liquid crystalline transition temperature of DMPC. Above LMPC/DMPC ratios of 3:2, the enthalpy of association was nearly zero. Gel filtration of the LMPC/DMPC mixtures at various molar ratios revealed the following: (1) Between 0 and 8 mole% LMPC, the LMPC simply enters the single- and multi-lamellar vesicles, but preferentially binds to the former; calorimetric measurements show that this reaction is exothermic at 23.7°; (2) At 12 mole% LMPC, both single- and multi-lamellar vesicles are converted to multibilayer vesicles containing LMPC. This interaction was exothermic; (3) Above 35 mole% LMPC, a new species whose size is between that of LMPC micelles and DMPC single bilayer vesicles, appears along with some multibilayer vesicles containing LMPC; this interaction was exothermic; (4) Above 50 mole% LMPC, increased amounts of mixed micelles of LMPC and DMPC were formed; the enthalpy of this reaction was zero. These results show that (1) single bilayer vesicles of DMPC more readily incorporate LMPC than the multi-lamellar type; (2) the association is most exothermic at T_c of DMPC; (3) the association is enthalpy driven at low mole% LMPC, but is entropy driven, presumably, through a hydrophobic effect at high mole% of LMPC.

W-PM-C10 DIRECT STRUCTURE DETERMINATION OF LIPID BILAYERS.
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Recently the use of direct methods of structure analysis using x-ray diffraction data from centrosymmetric membranes has provided unique and exact structural interpretation. The direct methods were limited to specimens having only a few unit cells, or where a continuous Fourier transform could be experimentally obtained in swelling experiments or from dispersions. In both cases, the autocorrelation function could be separated from the Patterson function. A direct analysis of the x-ray data from oriented lipid bilayers will be presented, assuming that the hydrocarbon regions in these systems act as a fluid layer in that it has an uniform electron density. Low-angle x-ray data from bilayers of lecithin (PC), phosphatidylethaneolamine (PE) and sphingomyelin (SM) and data for erythrocyte membranes and neutron diffraction data from PC were analyzed by this approach. This direct analysis at a resolution of about 5Å gives electron density profiles as previously deduced by other workers. An estimate of the width of polar regions is also obtained. The width of the polar regions in PC, PE and SM were found to be 22Å, 16Å and 20Å, respectively, in agreement with x-ray diffraction data from a homologue series of these compounds. This method may be useful in determining the phases of the x-ray reflections from lipid multilayers whenever the swelling method is inapplicable.

W-PM-C11 ORDERING AND DISORDERING EFFECTS OF MEMBRANE PERTURBERS IN VARIOUS LIPID BILAYERS.
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Recently we reported (Nature 263:253, 1976) that general anesthetics can selectively perturb lipid bilayer membranes. Thus while halothane and n-octanol fluidised all compositions of bilayer studied, pentobarbital ordered phospholipid bilayers and only became a membrane fluidiser when 20% cholesterol was incorporated in these bilayers. Here we examine further the hypothesis that the ability to order phospholipid bilayers requires that the perturber has a rigid structure. Membrane perturbations were studied by measuring the order parameter of spin-labeled phosphatidylcholine (7,6 PC) incorporated into lipid bilayers formed by vigorous agitation. The ability of five compounds with rigid ring systems to perturb both 96% egg phosphatidylcholine; 4% phosphatidic acid (PAPC) bilayers and 67% PAPC: 33% cholesterol bilayers was examined. The compounds were chlorpromazine, 3α and 3β-hydroxy-5β-pregnane-20-one, and 3α and 3β-hydroxy-5α-pregnane-11,20-dione. All these compounds increased the order parameter of the PAPC bilayers whilst decreasing the order parameter of the cholesterol containing membranes. Ethanol and butanol fluidised both these membranes. Thus, in addition to the apparent requirement for rigidity in the interface, structural features of the bilayer are required which are abolished by cholesterol incorporation. Other phospholipids may not share these features, for example preliminary results indicate that perturbers of this type fluidise phosphatidylserine bilayers and cause complex effects in dimyristoyl phosphatidylcholine.

W-PM-C12 A PHYSICAL THEORY OF GENERAL ANESTHESIA. Y. Katz* and S.A. Simon,* (Intr. by G. Padilla), Department of Physiology and Anesthesiology, Duke University Medical Center, 27710.

A theory has been developed that describes properties of the "site" of general anesthesia with thermodynamic parameters. The theory predicts a linear dependence of log Pan vs $(E_g)^{\frac{1}{2}}$ where Pan = anesthetic pressure and E_g = energy of vaporization of the gas. The slope and intercept of this line, constructed from experimental values, yields information about three parameters which describe the site general anesthesia; the cohesive energy, the interaction energy between the site and the anesthetic (adhesive energy), and the Barcley-Butler B coefficient. The values for these parameters are 13694 cal/mole, 299 $(cal/cm^3)^{\frac{1}{2}}$, and 0.00288°K^{-1} respectively. All of these values are very different than those for an organic liquid such as benzene or for a dimyristroyl lecithin bilayer above its transition temperature. A theoretical basis for selectivity was developed and served as a basis for comparison of selectivity between different systems. The selectivity coefficient obtained between anesthetic potency and inhibition of axon conductance was 0.86 for synapses it is 0.99 indicating that the synapse is better model to use for studing mechanisms of anesthesia. This work was supported by Grants HL-12157 and ONR N0014-67-A-0251-0022.

W-PM-C13 ELASTIC PROPERTIES OF ORIENTED MULTILAMELLA SAMPLES OF DIPALMITOYL-PHOSPHATIDYL-CHOLINE WITH AND WITHOUT CHOLESTEROL. J.P. LePesant* and P.S. Pershan, Division of Engineering and Applied Physics, Harvard University, Cambridge, Mass. 02138

The Brillouin scattering technique has been used to study the ultrasonic (~ 10^{10} Hz) elastic properties of single domain samples of (1) dipalmitoyl-phosphatidylcholine (DPPC) at varying water concentrations and temperatures and (2) DPPC-water samples containing 33 mole % cholesterol. At low water concentrations in pure DPPC samples, the elastic constant opposing changes in area per polar head group is considerably stiffer than the constant opposing changes in the interplanar spacing. For higher water these two elastic constants are comparable. For samples containing 33 mole % cholesterol the elastic constant opposing changes in interplanar spacing is larger than the one opposing changes in the area per polar head group. Differences between the elastic constant opposing changes in the area per polar head group at constant density in contrast to constant pressure will be discussed. The relationship between these measured elasticities and conventional elasticities of individual lipid monolayers and bilayers will also be discussed.

W-PM-C14 THE EFFECTS OF CHOLESTEROL AND SODIUM DODECYL SULFATE ON THE HYDRATION OF EGG YOLK PHOSPHATIDYLCHOLINE. R.P. Taylor and C. Huang, Biochemistry Department, University of Virginia School of Medicine, Charlottesville, Va. 22901.

Water which remains unfrozen at -25°C in the presence of phosphatidylcholine (PC) gives rise to a proton magnetic resonance signal which can be used to measure the hydration of single-walled vesicles and multilamellar liposomes of PC (R.P. Taylor, Arch. Biochem. Biophys. 173, 596 (1976)). At cholesterol to PC molar ratios below 35 mole %, the vesicle hydration signal consists of a relatively narrow symmetric peak (linewidth ∿ 150 Hz). At higher molar ratios, however, rather broad asymmetric signals appear (linewidths ∿ 300-1000 Hz) which indicate there must be at least two different types of slowly exchanging bound water associated with the lipid complexes. Though the shape of the hydration signal was qualitatively very different at high cholesterol to PC molar ratios for vesicles versus liposomes, in both cases two different types of bound water could be detected. It is possible to solubilize significant quantities of cholesterol by sonicating it in concentrated solutions of SDS. Addition of cholesterol to PC vesicles via these SDS-cholesterol complexes caused hydration changes in the PC, which, at high cholesterol to PC molar ratios, paralleled the effects of cholesterol on PC hydration in homogeneous vesicles (see above) in which the cholesterol and PC were simply cosonicated. The results suggest that at high cholesterol to PC molar ratios separate molecular domains exist in the bilayer, and that the exchange of water between the hydration sites in these domains must be slow at -25°C. (This work was supported by a Grant-in-Aid from the American Heart Association and with funds contributed in part by the Virginia Heart Association.)

W-PM-C15 COMPETITIVE DISSOCIATION OF $^{22}Na^+$ SORBED TO PHOSPHOLIPID MEMBRANES. A. Petkau and K. Black*, Medical Biophysics Branch, Whiteshell Nuclear Research Establishment, Atomic Energy of Canada Limited, Pinawa, Manitoba, Canada.

It is desirable that membrane transport theory take into account the kinetic behaviour of counterions at phospholipid bilayer interfaces with fixed dipoles. To this end, the rate of dissociation of $^{22}Na^+$ from model (soybean) phospholipid membranes (liposomes) was examined by flow dialysis (Colwick and Womack, J. Biol. Chem. 244 (1969) 774) using monovalent and divalent cations as competing ions. At 37°C, the estimated dissociation time (τ_d) for the competitive removal of 63% of the sorbed $^{22}Na^+$ by $^{23}Na^+$ varied little with the concentration of the latter. With Mg^{++} as the competing cation, τ_d decreased linearly from 36.9 ± 0.06 to 23.4 ± 0.7 min when the concentration of Mg^{++} was increased from 0.1 to 10 mM. At constant temperature and $[Mg^{++}]$, τ_d decreased exponentially with the amount of $^{22}Na^+$ sorbed. The activation energy for $^{22}Na^+$ desorption (E_a) by Ca^{++}, Mg^{++}, and $^{23}Na^+$ was 5.6 ± 1.2, 4.5 ± 1.0, and 2.9 ± 1.3 kcal/mole, respectively. Modification of the membranes with alamethicin, valinomycin, and gramicidin decreased E_a in relation to Ca^{++} from 5.6 ± 1.2 to 3.8 ± 1.6, 3.0 ± 1.8, and 3.8 ± 1.4 kcal/mole, respectively. By contrast, E_a in relation to $^{23}Na^+$ increased from 2.9 ± 1.3 to 4.1 ± 1.1 and 4.0 ± 2.0 kcal/mole with membranes modified by gramicidin and valinomycin, respectively. Ionizing radiation had a minor effect on the dissociation time.

W-PM-C16 MONOLAYER AT THE OIL/WATER INTERFACE AS A PROPER MODEL FOR BILAYER MEMBRANES.
S. Ohki, C. B. Ohki*and N. Duzgunes, Dept. of Biophysical Sciences, State University of New York at Buffalo, N.Y. 14226.

Surface tension measurements were made for phospholipid monolayers formed at the oil/water interface. It was found that the surface pressure of PS monolayer at hexadecane/water interface at 70$\AA^2$ per molecule was more than 40 dynes/cm. Interaction of water soluble proteins with the oil/water monolayer was studied in terms of the change in surface tension of the monolayer. The surface tension was reduced considerably upon introduction of proteins into the subphase solution (0.01M NaCl) at a low initial surface pressure less than 25 dynes/cm., but was not altered appreciably at a high initial surface pressure more than 40 dynes/cm. The phospholipid membrane conductance was increased when the water soluble basic proteins were placed on one side of the PS membrane only, but not altered when placed on both sides.

With the above experimental results and a theoretical consideration of physical states of lipid monolayer and bilayer membranes from their energetic points of view, it is concluded that the monolayer formed at the oil/water interface is a proper model system to represent the physical state of half of a bilayer in its liquid crystalline state.

W-PM-D1 PREDICTION OF PROTEIN SECONDARY STRUCTURE. P.Y. Chou* and G.D. Fasman, Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154

The predictive method of Chou and Fasman [Biochemistry 13, 222 (1974)] based on the known structure of 15 proteins has been refined, using the X-ray atomic coordinates and ϕ, ψ angles from 29 proteins. A refined set of P_α and P_β values for 20 amino acids have been obtained. The new values differ only for several residues: Met (P_α=1.20→1.45, P_β=1.67→1.05), Asn (P_β=0.65→0.89), and Asp (P_β=0.80→0.54). A total of 450 β-turns have been eluci dated in regions of chain reversals. Tetrapeptides whose C^α_i-C^α_{i+3} distances were below 7Å, and not in a helical region, were characterized as β-turns. An examination of the O_i-N_{i+3} distances of these β-turns showed that 53% of them were hydrogen-bonded. An environmental analysis of β-turn neighbor residues shows that reverse chain folding is stabilized by anti-parallel β-sheets as well as helix-helix interactions. The average frequency of β-turns is 30%, compared to the 38% for α-helices and 20% for β-sheets in the 29 proteins. Positional preferences of amino acids in β-turns as well as in boundary regions of helices and β-sheets have been determined and assist in obtaining higher predictive accuracy. Prediction of β-turns in homologous sequences of proteins shows conservation of β-turns despite variations of residues in the sequence. Predictions of regions containing both α- and β-forming residues may be resolved when the α and β conformational profiles are compared qualitatively and graphically. Additional conformational parameters of dipeptides and tripeptides are used to supplement the prediction based on single residue information. Preliminary computer predictions utilizing the above parameters improved the predictive accuracy.

W-PM-D2 CALCULATIONS OF CONFORMATIONAL ENERGY APPLIED TO THE RUBREDOXIN MOLECULE AND THE TRYPSIN INHIBITOR CRYSTAL. Jan Hermans, Dino Ferro,* Dept. of Biochemistry, Univ. of North Carolina, Chapel Hill, N.C. 27514 and Aneesur Rahman,* Argonne Natl. Lab., Argonne Ill.

Minimization of the conformational energy, with use of nonbonded potentials centered at all atoms and with inclusion of deformations of the molecular geometry, has been applied to two structures of rubredoxin, the structure S, fit to the multiple isomorphous replacement map and the structure LS, obtained by crystallographic refinement of structure S (Jensen and coworkers). The two energy minimized structures have strikingly similar conformation, energy and crystallographic R-factor (0.26 and 0.25, *vs.* 0.33 and 0.14 for S and LS). There is good agreement between structure LS and the structure obtained from it by energy minimization regarding the details of the active site conformation. The calculated and observed deviations from planarity of peptide groups correlate very well.

A molecular dynamics study of crystals of bovine trypsin inhibitor (PTI, Huber and coworkers) has been started. Approximately 500 water molecules were placed in the unit cell between the four symmetry related PTI molecules, and were then allowed to move independently in the force field of water and protein molecules. The water molecules behave in many ways as expected, forming an irregular hydrogen bonded network, attached with numerous hydrogen bonds to polar groups of the protein molecules. The equilibration is slow in the neighborhood of these polar groups, and the time average water distribution has not yet become similar in the four symmetrical parts of the unit cell. Simulated waters occupy sites identified crystallographically as preferred water positions with higher than average frequency. However, the average occupancy of these sites increases but slowly as the calculation progresses. The results of these calculations can be used to estimate the diffusion constants of the solvent.

W-PM-D3 LAC REPRESSOR: PEAK ASSIGNMENT USING SUPPRESSED MUTATIONS IN THE FLUORINE-19 NMR SPECTRUM OF THE 3-FLUOROTYROSINE SUBSTITUTED FORM. Maryann Jarema, Richard Friedman*, Jeffrey Miller*+ and Ponzy Lu, Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania, 19174 and +Department of Molecular Biology, University of Geneva, Geneva, Switzerland.

We have recently published the NMR spectrum of 3-Fluorotyrosine substituted lac repressor made by biosynthetic incorporation of the amino acid analogue (Proc. Natl. Acad. Sci. U.S., October, 1976).

Since we have a amber mutation at each of the eight tyrosine locations in the lac I gene, it is possible to systematically replace the tyrosines one at a time with several amino acids through nonsense suppression (Miller et.al. FEBS Proc. 38, 223, 1975). These suppressed mutations, when grown in the presence of 3-Fluorotyrosine, yield lac repressors missing one of the fluorines and thus a resonance peak in the NMR spectrum.

Several of the NMR spectra from these altered lac repressors will be presented along with the peak assignments to which they lead.

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W-PM-D4 METAL COMPLEXES OF HEW LYSOZYME. R.E. Lenkinski*, D.G. Agresti* and J.D. Glickson* (Intr. by G. Saccomani , University of Alabama in Birmingham, Birmingham, Alabama 35294; and University of Illinois Medical Center, Chicago, Illinois 60612.

Attention has recently been focused on the use of the trivalent lanthanides ions as probes in nmr studies of macromolecules. Previous analyses of the shifts induced by these paramagnetic ions have assumed that the magnetic susceptibility tensor of the metals is axially symmetric. This study has tested the validity of this assumption by analyzing reported nmr shift and relaxation data for various lanthanide complexes with lysozyme. For the Gd^{3+}-lysozyme [EC 3.2.1.17] complex the broadening data, fit to a $(1/r^6)$ model, places the metal ion in close proximity to the reported x-ray position. Contrary to current assumptions, the shifts induced by Nd^{3+} and Ce^{3+} exhibit considerable non-axial character. On the basis of a statistical hypothesis test, the axially symmetric model can be rejected with more than 97% confidence.

W-PM-D5 DYNAMIC PROBLEMS AT THE WATER-PROTEIN INTERFACE: RESULTS FROM NMR RELAXATION. E. Hsi and R. G. Bryant, Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455.

Nuclear magnetic resonance relaxation measurements of T_1, T_2 and $T_{1\rho}$ have been made on protein crystals, crosslinked protein crystals, and frozen protein solutions as a function of temperature and the mole fraction of protons in the water. The relaxation is complicated in that nonexponential decay of T_1 and T_2 is observed in many cases. In addition a significant dispersion in the longitudinal relaxation times is observed at frequencies below 1 MHz. This result and behavior of the relaxation as the concentration of protons in the sample is diluted with deuterons indicates that a new approach to understanding water relaxation and motion at the protein surface in these and other similar systems is required. A model which includes magnetic coupling of the water protons to the protein protons will be presented which may account for the nonexponential decay of longitudinal relaxation. The severe consequences of this coupling for making deductions about water molecule dynamics will be presented.

W-PM-D6 CONFORMATION OF ENKEPHALINS IN SOLUTION. M.A. Khaled, M.M. Long*, D.W. Urry, G.B. Brown*, and R.J. Bradley, Laboratory of Molecular Biophysics and the Cardiovascular Research and Training Center; and the Neurosciences Program, University of Alabama in Birmingham, Medical Center, Birmingham, Alabama 35294

Two endogenous pentapeptides, H-Tyr_1-Gly_2-Gly_3-Phe_4-Met_5, referred to as [Met^5] enkephalin, and H-Tyr_1-Gly_2-Gly_3-Phe_4-Leu_5, called [Leu^5] enkephalin, both with morphine-like action, have been studied in order to elucidate structure-function relationships. NMR methods have been used to investigate the conformational characteristics of the enkephalins in DMSO-d_6 solution. From the temperature dependence of the peptide NH protons and the spin pattern of Gly_2 and Gly_3CH_2 protons, it is indicated that both the compounds contain β-turns but that they differ in the amino acid-residues at the corners of the turn. In addition to a β-turn, other preferred secondary structures are indicated. NMR of [Leu^5] enkephalin gives evidence for a γ-turn and of [Met^5] enkephalin for a 7-atom H-bonded ring. Specific structural features of these molecules can be seen to resemble those of morphine and its derivatives and thereby provide insight into their morphine-like activity.

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W-PM-D7 DEUTERIUM AND CARBON-13 NMR OBSERVATIONS OF A PENTAPEPTIDE. George Neireiter, Jr.*, Bonnie Buchak*, Arthur O. Clouse*, and Frank R. N. Gurd, Department of Chemistry, Indiana University, Bloomington, IN 47401

The pentapeptide Gly-Leu-Ser-Glu-Gly is the N-terminal sequence of myoglobin from a common porpoise. It is also a good model compound for the N-terminal sequence of sperm whale myoglobin in which the amino terminal valine has been replaced by a glycine (Garner, W.H., and F.R.N. Gurd (1975) Biochem. Biophys. Res. Commun. 63: 262). The pentapeptide was synthesized by the solid phase technique using either carbon-13 or deuterium-enriched glycine at the amino terminus. By observing the deuterium or carbon-13 nuclear magnetic resonance signals, the pK of the amino terminus and data pertaining to overall and internal rotational motions of the peptide could be extracted. The pK could be monitored with either nucleus by observing the pH-dependent variance of the chemical shift or the spin-lattice relaxation time, T_1, for the enriched glycine. The quadrupolar coupling constant for deuterium could also be calculated at different pH values by comparing the relaxation equations for deuterium and carbon-13 and using the measured T_1 values. The rotational motion of the pentapeptide was treated by calculating an overall reorientation time, τ_R, using carbon-13 T_1 values of the backbone α-carbons. With this τ_R and the measured deuterium T_1, an internal rotational reorientation time, τ_G, could be derived. An estimate of the activation energy for rotation was obtained by monitoring the deuterium T_1 as a function of temperature. (Supported by PHS Grant HL 05556.)

W-PM-D8 PEPTIDE CONFORMATIONAL NMR STUDIES IN SOLUTION BY THE INTRAMOLECULAR NUCLEAR OVERHAUSER EFFECT (NOE). N.R. Krishna‡ S.L. Gordon‡ D.M. Chen‡ D.G. Agresti‡ J.D. Glickson‡ and R. Walter, Univ. Ala., Birmingham, Ala. 35294; Georgia Inst. Tech., Atlanta, Ga. 30332; Univ. Ill. Med. Ctr., Chicago, Ill. 60612.

Employing valinomycin as a model, we have demonstrated that the intramolecular NOE results from a predominantly dipole-dipole relaxation mechanism (BBRC 70, 746, 1976). The general theoretical equations for the dipolar NOE of a multispin system were derived from the Solomon equations. In contrast to the current treatment of NOEs of a multispin system, the newly developed formulation is applicable to arbitrary correlation times and resonance frequencies. A general matrix formalism for NOE conformational analysis of peptides, including the effects of conformational averaging, has also been derived. Evaluation of NOE data for valinomycin in dimethyl sulfoxide in terms of an approximate form of these equations indicates that of all the models proposed for the solution conformation of this antibiotic only the III-1 model of Patel and Tonelli (Biochemistry 12, 486, 1973) is consistent with the data. A rigorous evaluation of valinomycin NOE and ^1H spin-lattice relaxation time (T_1) data obtained in dimethyl sulfoxide and chloroform (free antibiotic and potassium complex) will be presented. These investigations illustrate that NOE and T_1 measurements in conjunction with values of vicinal coupling constants provide a very powerful method for conformational analysis of peptides. (Supported by grants AM-18399 and CA-13148).

W-PM-D9 NaCl EFFECT ON REPEAT PEPTIDES OF ELASTIN. M.M. Long,* D.W. Urry, D.W. Mason,* W.D. Thompson*. Laboratory of Molecular Biophysics and the Cardiovascular Research and Training Center, University of Alabama in Birmingham, Birmingham, Alabama 35294.

NaCl has a significant effect on both the intra- and/or interchain interactions of the repeat peptides of elastin; $(VPGG)_n$, 4n, $(VPGVG)_n$, 5n, $(APGVGV)_n$, 6n, synthesized in this laboratory. In normal saline, the PMR temperature coefficients for the 4n, 5n and 6n polymers decrease relative to their value in water; for the 4n and 5n the intramolecular effect is between 0-50°C, for the 6n, between 50°C-100°C. This indicates that the peptide NH's are more solvent shielded, i.e. more stably hydrogen bonded. Temperature profiles for coacervation of α-elastin, 5n, and 6n are also changed with NaCl, here, by altering the interchain interactions. Coacervation of α-elastin, 5n and 6n is a temperature ellicited, concentration dependent phase separation which involves an inverse temperature transition to increased peptide inter- and intramolecular order at higher temperatures. Normal saline shifts the temperature profiles in the direction of higher peptide concentration; for α-elastin and 5n, the curve is sharpened and moved to lower temperature; for the 6n the curve is translated to lower temperatures. NaCl titration of the 5n profile follows the pattern of increasing peptide concentration. $CaCl_2$ has the inverse effect. It is suggested that the NaCl effect could be understood in terms of a decrease in H_2O activity. (Supported by National Institutes of Health, Grant HL-11310).

W-PM-D10 ROTATIONAL MOTIONS IN BIOPOLYMERS. Richard J. Wittebort, Attila Szabo[‡], and Frank R. N. Gurd, Department of Chemistry, Indiana University, Bloomington, IN 47401

The measurement of molecular rotational rates by nuclear magnetic relaxation experiments is well-known. In the case of a biopolymer such as a protein or polypeptide complicated series of internal rotations can occur in the cases of an amino acid side chain or along the polypeptide backbone when secondary structural constraints are absent. Explicit expressions for analyzing relaxation rates and nuclear Overhauser enhancements in terms of the correlation times for the individual atomic rotations will be given. Several different models for rotational motions including random diffusion, restricted diffusion, and jumping between distinct sites will be discussed in terms of how the most appropriate description can be determined experimentally. Experiments on helical polypeptides such as poly-γ-benzylglutamate will be discussed as an example. (Supported by PHS Grants HL 05556 and T01 GM 1046.) ([‡]Supported by Petroleum Research Fund, American Chemical Society.)

W-PM-D11 FILTERING OF ARRAYS OF IMAGES IN RECIPROCAL SPACE (FAIRS); STRUCTURE INVESTIGATION OF VASOPRESSIN BY ELECTRON MICROSCOPY. J.W. Andrew*, F.P. Ottensmeyer, D.P. Bazett-Jones*, A.S.K. Chan*, and J. Hewitt*, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 500 Sherbourne St., Toronto, Ontario, Canada, M4X 1K9.

A major problem in observing the structure of biological molecules in the electron microscope is the fact that the electron dose required for high resolution electron microscopy generally results in serious radiation damage to the specimen. One solution to this problem for individual molecules has been to obtain several images of identical but different molecules at low dose and to superpose these images. This rather tedious process leads to an improvement in signal to noise of $\sqrt{N}$ for N different images of the same molecule in the same configuration. We have developed an improved procedure for signal to noise enhancement which combines the rapidity of optical treatment with the signal to noise enhancement which can be obtained from crystaline specimens. In this technique, images of individual molecules taken at low dose are accurately arranged into a two-dimensional square or rectangular array. Since this array in effect constitutes an artificial two-dimensional crystal, its optical diffraction pattern exhibits regular Bragg reflections which emphasize the structural similarities between one image and its regularly spaced neighbours. A filter consisting of a metal foil with a two-dimensional regular array of perforations at the positions of the Bragg reflections is then placed in the diffraction plane. The filter allows practically all of the signal, which is regularly repeated in the array of images, to be transmitted, while most of the noise is blocked by the foil. The signal to noise enhancement in the resulting averaged and filtered image is found to approach $N^{3/2}$ for N images in the unfiltered array. We illustrate this technique which we call FAIRS (Filtering of Arrays of Images in Reciprocal Space) with dark field micrographs of the cyclic polypeptide vasopressin.

W-PM-D12 VIBRATIONAL ANALYSIS OF SIDE-CHAIN ROTATIONAL ISOMERISM IN β-POLY(L-VALINE). W.H. Moore* and S. Krimm, Biophysics Research Division, Institute of Science and Technology, University of Michigan, Ann Arbor, Mich. 48109.

We have calculated the normal vibration frequencies of poly(L-valine), PLV, in the antiparallel chain pleated sheet structure for C^{α}-C^{β} torsion angles, χ_1, of 60°,180°, and 300°. (Energy calculations on a single chain[1] have indicated that all three rotational states are energetically possible.) The vibrational force field is that which we have developed for β polypeptides[2]. The calculations show that the frequencies in the amide III region and those below 700 cm^{-1} are significantly different for the three isomers. A structure near χ_1 = 180° gives best overall agreement with the observed infrared and Raman bands. Comparison with the observed spectra further indicates that the χ_1 = 60° structure is not normally present in β-PLV. We find, however, that this structure has characteristic bands near those at 543 and 414 cm^{-1} observed in nujol mulls of as-polymerized PLV. This suggests that side-chain rotational isomerism can account for these bands, and it is unnecessary to invoke local α-helical conformations to explain their presence[3].

This research was supported by grants from the National Science Foundation.

1. T. Ooi, et al, J. Chem. Phys. 46, 4410 (1967)
2. W.H. Moore and S. Krimm, Biopolymers, in press
3. K. Itoh and G.D. Fasman, Biopolymers 14, 1755 (1975)

W-PM-D13 NMR EVIDENCE FOR A 1:1 COMPLEX BETWEEN SULFUR-CONTAINING AND AROMATIC MOLECULES. B. L. Bodner*, L. M. Jackman*, and R. S. Morgan, Department of Biochemistry & Biophysics, and Department of Chemistry, The Pennsylvania State University, University Park, PA 16802.

Morgan et al.[1] have called attention to the clusters of sulfur-containing and aromatic residues which exist in the proteins, lysozyme, trypsin inhibitor, and ribonuclease. To learn more about possible complex formation in solution between sulfur-containing and aromatic molecules, the nuclear magnetic resonance spectra of mixtures of dimethyl disulfide, DMDS, (or dimethyl sulfide, DMS) and benzene were obtained. We found that the resonance frequency of the methyl protons is shifted upfield in the presence of benzene. By measuring these chemical shifts as a function of the mole fraction of the benzene in these mixtures, and applying the Method of Continuous Variations (or Job Method), we find that a 1:1 complex between these two species exists. In this complex, the DMDS (DMS) aligns itself near the benzene ring so that the methyl protons are shielded by the ring's field from the external field. We chose DMDS (DMS) and benzene to serve as a model system for amino acid interactions in proteins: the DMDS and DMS being similar to Cysteine and Methionine and the benzene being analogous to the aromatic side chains of Tyrosine, Phenylalanine, etc. Because we have found that sulfur molecules form complexes with the Pi electrons of aromatic systems, we infer that this specificity may also occur in proteins and may serve as a mechanism which helps determine conformation.

[1] Abstract B5, Clemson meeting of the American Crystallographic Association, January, 1976.

W-PM-D14 PROTON SPIN-LATTICE RELAXATION IN DEOXYMYOGLOBIN.‡ W. T. Dixon* and O. Lumpkin*† (Intr. by B. Zimm) Physics Department, University of California, San Diego, La Jolla, Ca. 92093

We have measured proton spin-lattice relaxation times in deoxymyoglobin (Fe^{2+}, S = 2) at temperatures between 1.5° and 4.2°K and in applied magnetic fields from 0 to 2 kG. The results indicate that (1) the electronic spin-lattice relaxation time is of order 10^{-5} sec, and (2) the interval between the lowest electronic spin-state and the next higher spin-state is at most 2 cm^{-1}. This latter result appears to disagree with paramagnetic susceptibility studies on the same molecule.[1]

1. N. Nakano, J. Otsuka, and A. Tasaki. Biochim. Biophys. Acta 278, 355 (1972).

‡Work supported by the National Science Foundation

†Alfred P. Sloan Foundation Fellow

W-PM-D15 PROTON RELAXATION STUDIES OF THE INTERACTION OF SMALL MOLECULES WITH CYTOCHROME P450. S.B. Philson‡ P.G. Schmidt, P.G. Debrunner, and I.C. Gunsalus‡ Department of Physics and School of Chemical Sciences, University of Illinois, Urbana, IL 61801.

The longitudinal and transverse relaxation times of water protons in solutions of bacterial cytochrome P450 in its native, low spin ferric form have been measured. From the frequency dependence of the relaxivity an electron spin relaxation time for the heme iron of 4×10^{-10} sec is obtained. The temperature dependence suggests a transition from a slow to a fast exchange region, and thus makes possible the estimation of the exchange rate (3.6×10^5 sec^{-1} at 25°C, E_a = 13 kcal/mole), the Fe-proton distance (2.75Å), and the hyperfine coupling constant (2.8×10^6 Hz). These estimates assume that two protons per P450 molecule are exchanging; they are consistent with the presence of a water molecule as a ligand of the heme. In addition, proton relaxation data for substrates and inhibitors in the presence of P450 are discussed.

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W-PM-D16 NUCLEAR MODULATION STUDIES OF TYPE I AND TYPE II COPPER IN RHUS VERNICIFERA LACCASE. J. Peisach, W. B. Mims*, M. T. Graziani* and B. Mondovi* , Albert Einstein Col. of Medicine, Bronx, N.Y., Bell Labs, Murray Hill, N.J. and University of Rome, Italy.

A pulsed EPR method has been used to study the coupling between electron spins and the nuclear moments in nearby atoms. This coupling is observed by recording the electron spin echo envelope, i.e., the function obtained by plotting the electron spin echo amplitude against the time between spin echo generating pulses. Instead of decaying monotonically the envelope contains periodic components which arise from electron nuclear coupling between paramagnetic centers and close-lying atomic nuclei. The frequencies of the periodic components belong to superhyperfine structure of the electron paramagnetic resonance and are characteristic of the particular interacting nuclei. The depth of modulation is directly proportional to the number of nuclei and inversely proportional to the sixth power of the distance between the electron and nuclear paramagnets. The electron spin echo envelope for Type II (non-blue) copper of laccase was studied at a Zeeman field where Type I (blue) copper does not contribute to the EPR, i.e., in the region of the low field hyperfine line at $g_{\parallel}$. The envelope showed a frequency characteristic of protons and, in addition, features arising from electron nuclear coupling with remote ^{14}N of imidazole. Other experiments were performed on laccase from which Type II copper was removed reversibly and in which the Type I copper retains its optical and magnetic properties (Graziani et al., FEBS Letters, in press). Here too, coupling with protons and with ^{14}N of imidazole was indicated. Thus, both Type I and Type II copper of laccase are ligated to histidyl residues of the protein.

W-PM-E1 AN OMEGA PROTEIN FROM MICROCOCCUS LUTEUS. V.T. Kung* and J.C. Wang, Chemistry Department, University of California, Berkeley, CA 94720

Omega proteins are a class of enzymes which can introduce transient breaks into a DNA, and are usually assayed by their abilities to remove superhelical turns of a covalently closed DNA duplex. The first omega protein isolated, that of Escherichia coli, requires Mg(II) and a DNA substrate which is negatively superhelical. All other such proteins isolated to date are from eukaryotic cells. They do not require Mg(II) and can act on either negatively or positively twisted DNAs. To test whether there are general differences between such proteins from prokaryotic and eukaryotic cells, we have purified the omega protein of Micrococcus luteus to homogeneity. The protein is a single polypeptide of molecular weight 120,000, with an acidic isoelectric point. It requires Mg(II), catalyzes the removal of negative superhelical turns efficiently. With positively twisted DNA the reaction is very slow. Similar to the coli enzyme it also catalyzes the formation of knotted single-stranded rings, and can form an intermediate with the protein covalently linked to the 5' end of a DNA strand.

W-PM-E2 PURIFICATION AND PROPERTIES OF A VACCINIA VIRUS DNA NICKING-CLOSING ENZYME. P.D. Foglesong‡ E.C. Ressner‡ and W.R. Bauer, Department of Microbiology, School of Basic Health Sciences, State University of New York, Stony Brook, N. Y. 11794.

Vaccinia virus cores contain a DNA nicking-closing enzyme which differs from the corresponding host enzyme in salt optimum, sedimentation coefficient, and polypeptide composition as determined by SDS-polyacrylamide gel electrophoresis. The nicking-closing activity of both intact vaccinia cores and of highly purified enzyme accepts either positive or negative superhelical DNA as substrate. Both viral PM2 and plasmid PSM-1 DNAs have been used to assay nicking-closing activity by agarose gel electrophoresis and by ethidium bromide fluorescence. A fluorescence method has been developed to determine the fraction of closed circular DNA converted to nicked or linear forms in the course of the assay by comparing the fluorescence intensities at pH 7.0 and pH 12.0. These assay methods have made possible extensive purification of the enzyme by chromatography on denatured DNA cellulose and hydroxyapatite. Enzyme preparations containing 0.33 M potassium phosphate, pH 7.5, are stable for several weeks when quickly frozen and stored at -20°C. The most highly purified preparation contains two polypeptides: a 24,000 dalton protein which is a major component of the virus (7% by weight) and a 35,000 dalton protein which is present in the virion in small amounts (0.2%). The 35,000 dalton component appears to be necessary, but not sufficient, for optimal enzyme activity. The velocity of the nicking-closing reaction has been determined as a function of enzyme concentration and follows a sigmoidal relationship, suggesting that subunit association is required for optimal activity. The reduction in activity which accompanies dilution of the enzyme may be understood on a similar basis.

W-PM-E3 VISUALIZATION OF DRUG-NUCLEIC ACID INTERACTIONS AT ATOMIC RESOLUTION: UNIFYING STRUCTURAL CONCEPTS IN UNDERSTANDING DRUG-DNA INTERACTIONS. S. C. JAIN, C. C. TSAI and H. M. SOBELL, Department of Chemistry and Radiation Biology and Biophysics, University of Rochester, Rochester, N.Y. 14627

We have determined the three dimensional structures of two crystalline complexes of ethidium with 5-iodocytidylyl(3'-5')guanosine and 5-iodouridylyl(3'-5')adenosine by X-ray crystallography. These structures demonstrate drug intercalation into miniature Watson-Crick double helices. The stereochemical information afforded by these studies have enabled us understand how ethidium binds to DNA. Major features of the ethidium-DNA model are: a. Intercalation of the phenanthridinium ring system of ethidium molecule between base-paired dinucleotide sequences in DNA and the utilization of symmetry in the interaction. The phenyl- and ethyl- groups of ethidium molecule lie in the narrow groove of the DNA double helix. b. unwinding of DNA at the immediate site of intercalation by about -26°. This reflects specific conformational changes, primarily glycosidic torsional angles describing the base-sugar orientation and the following sugar ring puckering pattern: C3' endo (3'-5') C2' endo at the intercalation site. Helix axes of B-DNA sections above and below the ethidium molecule are displaced by about +1.0 Å; this reflects the realization that drug intercalation into DNA is accompanied by helical screw axis dislocation in its structure, a variable whose magnitude determines the relative ring overlap between an intercalative drug molecule and adjacent base-pairs. c. intercalative binding limited to every other base-pair at maximal drug-nucleic acid binding ratios(i.e., a neighbor exclusion model); this reflects the stereochemical constraints imposed by the sugar-puckering pattern at the immediate site drug intercalation. These structural informations allow us to understand the detailed nature of actinomycin-DNA and irehdiamine-DNA binding and will be described at the meeting.

W-PM-E4 EFFECT OF HYPERTHERMIA ON NONHISTONE PROTEINS ISOLATED WITH DNA. S.P. Tomasovic*, G.N. Turner*, and W. C. Dewey, Department of Radiology & Radiation Biology, Colorado State University, Fort Collins, Colorado 80523.

Hyperthermic treatment of CHO cells at 45.5°C resulted in an increase in nonhistone protein isolated with DNA. The increase, as measured by ^{3}H-tryptophan to ^{14}C-thymidine ratios, was proportional to heat duration and was 1.6 times the control after 15 minutes of heating. Irradiation (up to 3000 rads) had no effect on these results. If cells were incubated at 37°C after heating, recovery to control levels occurred within 12 hours. Analysis of labeled protein, after SDS slab gel electrophoresis, showed no consistent density or radioactivity differences in individual bands after heating. However, there was a nonspecific overall increase in high molecular weight bands in heated samples. These results were independent of chromatin isolation technique (Zubay-Doty isotonic versus Hancock hypotonic) and of culturing conditions (monolayer versus suspension).

W-PM-E5 INCREASED BINDING OF CHROMOSOMAL PROTEINS INDUCED BY HYPERTHERMIA. J.L. Roti Roti, S. Cohen*, and M.L. Lynch*, Department of Radiology, University of Utah, Medical Center, Salt Lake City, Utah 84132

The effects of hyperthermic treatment on chromatin structure of mammalian cells are being investigated. When chromatin is isolated from heated HeLa cells (45°C, 30 minutes) the protein to DNA ratio is increased by a factor of 1.23 $\pm$ .05 relative to that from unheated cells. The increase in protein to DNA ratio of chromatin is heat dose dependent. Chromatin is isolated from clean nuclei (< 1% with cytoplasmic taps) by homogenization in distilled water. Nuclei were obtained by successive Triton-X100, EDTA washes. The following experiment is designed to investigate qualitatively and quantitativity the heat-induced increase in chromosomal protein. HeLa cells were labeled for 15 hours with either ^{3}H-leu. (8 x 10^{7} cells) or ^{14}C-leu. (4 x 10^{7} cells). One-half of the ^{3}H-labeled cells were heated in a water bath at 45°C for 30 minutes. The unheated cells are divided into 4 control samples (@ 2 x 10^{7} cells; two are ^{3}H-labeled and two are ^{14}C-labeled) and 2 heated samples (both ^{3}H-labeled). Chromatin is isolated directly from two of the ^{3}H labeled samples (one heated and one unheated). The remaining samples are mixed, prior to chromatin isolation, in two combinations: 1) heated, ^{3}H-labeled with unheated ^{14}C-labeled; and 2) unheated, ^{3}H-labeled with unheated, ^{14}C-labeled. After isolation, the chromosomal proteins are separated on SDS polyacrylamide gels. The results of these studies suggest: 1) The heat induced increase in chromosomal proteins is due to increased binding of nonhistone type chromosomal proteins. 2) The increase in nonhistone chromosomal protein (NHCP) fraction is due to a general increase of NHCP across the molecular weight spectrum.

W-PM-E6 CATALYSIS OF DNA STRAND REASSOCIATION BY THE E.COLI DNA BINDING PROTEIN. C.H. Christiansen, Department of Biochemistry, Stanford University, Stanford, CA 94305

E.coli DNA binding protein shares functional and physical properties with T4 gene 32 protein; both proteins are essential for DNA replication, both bind tightly and cooperatively to single-stranded DNA, and both can under certain conditions denature native DNA. Gene 32 protein can catalyze DNA strand reassociation but this property has not yet been demonstrated for the E.coli DNA binding protein. We have adapted an S1 endonuclease assay for measuring DNA reassociation in the presence of DNA binding protein and have found conditions where strong catalysis of DNA reassociation is observed. Under optimal conditions, adding a stoichiometric amount of binding protein (10 x DNA, w/w) gives a 200-fold increase in the rate of DNA reassociation. These conditions are 0.04 M Mg^{++}, 0.01 M Na^{+}, 37°C, and pH 6.0. The enhancement increases as the concentration of Mg^{++} is raised from about 1 mM, and 0.04 M Mg^{++} can be substituted by 1-3 mM spermidine. The enhancement of reassociation rate decreases drastically at pH values above 6.0 and is virtually 0 above pH 7.0. The degree of enhancement increases with the length of the DNA strands, but some enhancement is observed even at strand sizes of 200-300 bases. Our result extends the list of homologies between gene 32 protein and E.coli DNA binding protein to include the ability to catalyze DNA reassociation. The conditions found optimal for this catalytic activity resemble physiological conditions more closely than those for which unwinding of the double helix of native DNA has been reported. Studies of the mechanism by which the binding protein catalyzes DNA reassociation are in progress.

W-PM-E7 THE INFLUENCE OF PROTEIN-DNA COMPLEXES ON THE STABILITY OF ADJOINING DNA REGIONS
R. M. Wartell, School of Physics, Georgia Institute of Technology, Atlanta, Ga

Theoretical calculations were made to examine the influence of melting and stabilizing proteins on the thermal stability of adjoining base pairs (bp). A DNA sequence 1000 bp. long containing the 123bp lactose operon in the center was employed as a model system. Melting curves of base pairs near the sites for lac repressor, RNA polymerase, and CAP protein were calculated in the presence and absence of each protein. Transmission of stability or instability (telestability) to a base pair was quantified by the difference in melting temperatures, t_m, with and without each protein. The modified Ising model was used to calculate the transitions. Calculations showed that a melting protein at the CAP site decreases the t_ms of base pairs 18 to 120bp away by 0.92°C to 0.77°C. The free energy required to melt the DNA region 20-60bp. away is lowered by about 0.8k cal. A melting protein at the transcription initiation site lowers the t_m of the base pairs 18bp away by 2.5°C in the direction of transcription and by 1.0°C in the opposite direction. The base pair compositions of the protein site and the region surrounding the site strongly influence the magnitude of the t_m change. G.C rich regions intervening between a protein complex and the base pair being examined decrease the t_m change, whereas A.T rich regions increase the change. The opposite effects are observed when the composition of the protein site is examined. The largest t_m change occurs when the protein site is G.C rich. The above results, taken together with stopping sequences of RNA polymerase, suggest a model for RNA polymerase movement which will be discussed. Supported by NSF.

W-PM-E8 MACROMOLECULAR ORGANIZATION OF RNA IN SMALL RIBOSOMAL SUBUNIT. A. A. Bogdanov* (Intr. by E. W. Westhead), Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow, U.S.S.R., and Department of Biochemistry, University of Massachusetts, Amherst, Mass. 01003.

Measurements of the coefficients of sedimentation of 16S RNA and 30S subunits of E.coli ribosomes over a wide range of Mg^{++} and K^{+} concentrations indicate that RNA in small ribosomal subunit has in physiological conditions very compact and contracted conformation. Studies of RNA properties in a ribonucleoprotein particles with different protein composition by means of various approaches (kinetics of RNase hydrolysis, fluorescent dyes binding, chemical modification, circular dichroism, complementary oligonucleotide binding, sedimentation analysis) show that only a few proteins, such as S4, S7 and S15, cause the most drastic changes in RNA conformation. These proteins are suggested to induce the specific RNA-RNA interactions in 16S RNA macromolecule which are primarily responsible for organization of the compact structure of ribosomal subunits. The last conclusion follows from the fact that 30S and 50S subunits with cross-linked neighboring proteins and fixed RNA-protein contacts undergo the unfolding in Mg^{++}-free solutions exactly as the subunits without cross-links. The existence of the protein-induced contracted structure of RNA in subunits may be directly related to their conformational changes during the ribosomal cycle.

W-PM-E9 PHOTOREACTIVATING ENZYME: THE ACTION SPECTRUM FOR REVERSAL OF THE COMPLEX OF ENZYME AND IRRADIALED DNA IS SIMILAR TO THE ACTION SPECTRUM FOR DIMER MONOMERIZATION. K.L. Wun* and J.C. Sutherland, Department of Physiology, College of Medicine, University of California, Irvine, CA 92717.

When photoreactivating enzyme, purified from E. coli, is mixed with uv irradiated DNA, hyperchromicity is observed for wavelengths between 300 and 450 nm. There is concomitant hypochromicity at wavelengths less than 300 nm (1). Irradiation at 365 nm reverses both the long wavelength hyperchromicity and short wavelength hypochromicity with first order kinetics (2). Measurement of the reversal induced by other wavelengths (313, 334, 405, 436 and 546 nm) also reverses the hypo- and hyperchromicity with first order kinetics. The photochemical rate constants obtained from the experiments determine the action spectrum for complex reversal. The maximum effect occurs at 365 nm and decreases monotonically with both increasing and decreasing wavelengths. Thus, the shape of this action spectrum is similar to the shape of the hyperchromicity observed for λ>300 nm (1) and to the action spectrum for the photoenzymatic monomerization pyrimidine dimers. This result supports the hypothesis that the hyperchromic band, which appears when PRE is mixed with uvDNA, is responsible for the absorption of photoreactivating light.

(1) K.L. Wun and J.C. Sutherland (1976) Abstracts of the 4th annual meeting of the Am. Soc. for Photobiol., Denver, Co., p45.

(2) J.C. Sutherland and K.L. Wun (1976) Abstracts of the 6th International Cong. on Photobiol., Rome.

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W-PM-E10 THE NONESTERIFIED CHOLESTEROL BINDING PROTEIN FROM HUMAN URINE.
A. Rosenthal and R. Mitchell*, Department of Laboratory Medicine, Allegheny General Hospital, 320 E. North Ave., Pittsburgh, Pa 15212

Urine from persons with carcinoma of the steroid producing glands or their target organs is often rich in nonesterified cholesterol(NEC) all of which is bound to a protein(NECBP). This protein has been found to undergo dissociation from its dominant for(molecular weight greater than 5×10^5) to species with molecular weights of approximately 2×10^5 and 5×10^4. Repeated molecular sieve chromatography of the high or low molecular weight fractions does not totally separate either form, strongly suggesting a self-association. Prior enrichment of the urine with NEC appears to produce a small shift to the higher molecular weight. Preliminary sedimentation velocity analyses have shown an higher molecular weight species which sediments very rapidly during acceleration with excessive boundary spreading. Polyacrylamide gel electrophoresis of normal urine, NEC rich urine, and the three major fractions of the NEC rich urine indicate a single component for the higher molecular weight species. This same component corresponds to one of the three bands present in the lower molecular weight fraction. This band is also present in the NEC rich urine but is not detectable in normal urine.

W-PM-F1 X-RAY ABSORPTION EDGE STUDIES ON OXIDIZED AND REDUCED CYTOCHROME C OXIDASE. V.W. Hu*, and S. I. Chan, Department of Chemistry, California Institute of Technology, Pasadena, CA 91125; G.S. Brown*, Bell Telephone Laboratories, Murray Hill, N.J. 07974.

The X-ray absorption edge spectra of oxidized and reduced cytochrome c oxidase have been obtained using synchrotron radiation from the Stanford Positron Electron Accelerating Ring at the Stanford Linear Accelerator Center. Using this intense source of tunable X-irradiation, both the copper and iron K-edges of the protein have been detected. Upon reduction with dithionite, both edges are shifted to lower energies, reflecting the change in the metal centers to lower oxidation states. A number of model copper compounds in various oxidation states have also been examined. By comparison of the positions and intensities of the peaks in the oxidase spectra with those of the model compounds, tentative assignments have been made for the peaks in the complex oxidase spectra. The data on the Cu K-edge of the oxidized protein are best interpreted in terms of two non-equivalent copper ions in the +2 oxidation state. One of the coppers seems to be very covalent. Both of the copper ions appear to be reduced to Cu^{+1} in the dithionite-treated oxidase. The shift in the Fe K-edge of cytochrome oxidase upon reduction is very small and is similar to that previously observed by Shulman et al.[1] for the heme iron of cytochrome c.

[1]Shulman, R.G., Yafet, Y., Eisenberger, P., Blumberg, W.E., Proc. Natl. Acad. Sci. USA, 73, 1384 (1976).

W-PM-F2 THE STRUCTURE OF A CYTOCHROME OXIDASE/LIPID MODEL MEMBRANE J. K. Blasie, M. Erecinska, J. S. Leigh and S. Samuels*, Dept. of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pa. 19174

Membranous cytochrome oxidase prepared from pigeon breast mitochondria essentially by the method of Sun, et al. typically possessed lipid/protein molar ratios of 20-30/1. Dispersions of this membranous cytochrome oxidase containing minimal amounts of detergent were formed into oriented multilayers by centrifugation followed by slow partial dehydration at 4°C. Lamellar x-ray diffraction from such hydrated oriented multilayers evidenced a mosaic spread of less than 15°, some lattice disorder ($\gamma/D \sim 0.09$) and extended out to $(2 \sin\theta)/\lambda \sim 1/6$Å. This lamellar x-ray diffraction has been analyzed using direct methods to provide the electron density profile for this membrane to ~8Å-resolution. Equatorial x-ray diffraction is observed arising from the packing of oxidase molecules in the plane of the membrane and is characteristic of a non-crystalline planar arrangement of the oxidase molecules. This equatorial diffraction possesses strong components in the region of $(2 \sin\theta)/\lambda \sim 1/10$A which together with the strong diffraction at $(2 \sin\theta)/\lambda \sim 1/5$Å on the lamellar axis suggests the presence of bundles of α-helices within the oxidase molecules whose average orientation is normal to the membrane plane. ESR studies of these oriented multilayers of membranous cytochrome oxidase at ~10°K show that low-spin forms of the oxidase hemes are oriented such that the normal to the heme plane lies predominantly in the membrane plane. Polarized optical spectroscopy of these oriented multilayers is also in progress. We note that lamellar x-ray diffraction from oriented multilayers of the crystalline form of membranous cytochrome oxidase (Vanderkooi, et al.) could in principle be combined with electron diffraction from tilted bilayers of the crystalline oxidase (Henderson, et al.) to provide a complete three-dimensional structure for the cytochrome oxidase molecule.

W-PM-F3 THERMODYNAMIC AND SPECTRAL PROPERTIES OF THE HEMES AND COPPERS OF CYTOCHROME OXIDASE: A STUDY BY MAGNETIC CIRCULAR DICHROISM AND ELECTRON PARAMAGNETIC RESONANCE. Gerald T. Babcock, Larry E. Vickery*† and Graham Palmer*†, Chemistry Department, Michigan State University, East Lansing, MI 48824, †Chemistry Department, University of California, Berkeley, CA 94720 and ‡Biochemistry Department, Rice University, Houston, TX 77005.

Reductive titrations of cytochrome oxidase have been carried out under inert and carbon monoxide atmospheres using optical, EPR and MCD spectroscopies to monitor spectral changes in the oxidase as a function of electrons added. The concentrations of cytochrome a^{2+} and cytochrome a_3^{2+} at each point in the titration could be determined by deconvolution of the MCD spectra; at all stages of reduction the concentrations of the species giving rise to the g=2 (Cu^{2+}), g=3 (low spin Fe^{3+}) and g=6 (high spin Fe^{3+}) EPR resonances were quantified. At half reduction of the oxidase in the absence of added ligands roughly 45% of the a and 55% of the a_3 are reduced. During the titration the g=3 spin concentration decreases more rapidly than the cytochrome a^{2+} concentration increases. However, in this region the g=6 spin concentration increases and the sum of the concentrations for the g=3 species, the g=6 species and cytochrome a^{2+} accounts for one heme a per oxidase at all stages of reduction. In titrations carried out in the presence of CO the redox properties of cytochromes a and a_3 are markedly altered. The a_3^{2+} CO complex is fully formed prior to the reduction of either cytochrome a^{3+} or the EPR detectable Cu^{2+}. These latter two species subsequently titrate with cytochrome a^{3+} exhibiting a redox potential 45 mV more positive than Cu^{2+}. The g=3 EPR signal attributed to cytochrome a^{3+} declines as the MCD intensity of cytochrome a^{2+} increases. No significant high spin (g=6) intensity is observed at any intermediate stage of reduction. (This research was supported by NIH Grants GM-21337 and HL-02052, the Welch Foundation (C636) and by the Energy Research and Development Administration).

W-PM-F4 DIRECT EXPERIMENTAL EVIDENCE OF VIBRONICALLY COUPLED ELECTRON TUNNELING IN BIOMOLECULES.[+] M. J. Potasek* and J. J. Hopfield, Joseph Henry Laboratories of Physics, Princeton University, Princeton, N. J. 08540 and Bell Laboratories, Murray Hill, N. J. 07974

Direct evidence for vibronically coupled electron tunneling as the fundamental mechanism of electron transfer in photosynthesis and in respiration is presented. Using a novel technique of excitation modulation spectroscopy, we study electron tunneling at room temperature between cytochrome c and ferrihexacyanide. The data are in agreement with the Hopfield hypothesis that, in biological systems, electrons transfer by tunneling at all temperatures and the transfer distance remains constant. These experiments test the general constructs of the Hopfield model of vibronically coupled transfer and rule out large transfer distances. Traditional techniques of spectroscopy are not feasible for these measurements. Our technique of excitation modulation spectroscopy has the sensitivity necessary to measure induced concentration changes in cytochrome c down to a level of 10^{-11} molar and can simultaneously examine the kinetics of electron transfer on a submillisecond scale. To understand the mechanism of electron transfer in biomolecules is of fundamental importance when considering the interaction of components in a complicated electron transfer chain.

[+]Work at Princeton supported in part by NSF Grant DMR 75-14264

W-PM-F5 MECHANISMS OF ELECTRON TRANSFER BY C-TYPE CYTOCHROMES. M. A. Cusanovich, Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Previous studies in this laboratory (Bioinorganic Chem. 4, 337 (1975)) have shown that Rhodospirillum rubrum cytochrome c_2 undergoes rapid oxidation and reduction by the iron hexacyanides. These reactions are kinetically complex with available evidence consistent with oxidation and reduction taking place at a common site on the surface of the cytochrome which has a net positive charge. We have recently extended these studies to a wide range of reactants including: SO_2^-, $S_2O_4^=$ ascorbate, oxidized azurin and reduced azurin. Based on an analysis of the kinetics of oxidation and reduction with the different reactants, as a function of temperature, pH and ionic strength, it appears that the same site of electron transfer on the proteins surface is operative in all cases. The data presented can be interpreted in terms of three ionizable amino acid side chains participating in the electron transfer process in each oxidation state. Based on the pK values determined and available structural information the nature of the specific residues involved will be discussed. Finally, the studies with iron hexacyanides have been extended to cytochrome c_2 from four other sources (R. vanniellii, R. capsulata, R. palustris and R. sphaeroides). This work coupled with available structural and sequence information permits speculation as to the structure of the site of electron transfer on cytochromes c_2 and by analogy mammalian cytochrome c. This research was supported by NIH Research Grant GM-21277 and NSF Grant PCM 75-21009.

W-PM-F6 OPTICAL AND MAGNETIC PROPERTIES OF FERRIC HEME OCTAPEPTIDE COMPLEXES FROM CYTOCHROME C. E.K. Yang, L.E. Vickery, and K.H. Sauer, Department of Chemistry and Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California Berkeley, Calif. 94720.

Complexes of the NH_2-acetylated heme octapeptide (N-H8PT) from horse heart cytochrome c[1] with CN^-, Imidazole, N_3^-, H_2O and F^- in 0.1M phosphate, pH 7.0, 20.0°C exhibit absorption spectra similar to those of hemeprotein derivatives and their paramagnetic susceptibilities are as predicted by ligand field strength considerations. The complex of the N-H8PT with N-acetylated methionine, however, which is analogous to cytochrome c in its axial iron coordination, yields a spectrum containing a residual of the charge transfer band near 625 nm, characteristic of high spin heme, in addition to the low spin features characteristic of cytochrome c. Measurement of paramagnetic susceptibility of the methionine complex by the NMR technique[2] is consistent with the absorption result indicating the presence of a high spin (S=5/2) $\rightleftarrows$ low spin (S=1/2) equilibrium. Analysis of the temperature dependence of χ_o yields thermodynamic parameters for the transition between the two spin states of ΔH^o = -8.0 Kcal/mole and ΔS^o=-26.4 e.u. These results suggest that in native cytochrome c the folding of the polypeptide chain, and not simply the combined axial ligand field strength of the methionine thioether sulfur and imidazole nitrogen, is required to shift the equilibrium toward the low spin form.

1. H.Tuppy and S.Paleus, Acta. Chem. Scand., 9, 353 (1955).
2. D.F.Evans, J.Chem. Soc., 2003 (1959).

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W-PM-F7 DETERMINATION OF IRON LIGAND FIELD SYMMETRIES IN REDUCED CYTOCHROMES BY HIGH RESOLUTION RESONANCE RAMAN EXCITATION PROFILES (RREP). J. M. Friedman,* D. L. Rousseau Bell Laboratories, Murray Hill, N. J. 07974, and F. Adar, Johnson Foundation, University of Pennsylvania, Philadelphia, Pa. 19174.

By examining the resonance Raman emission and relaxed fluorescence originating from excitations in the vicinity of the split α band ($g_0 \xrightarrow{h\nu} Q_0$) of ferrocytochromes c and b_5 at 6°K we conclude that the absorption line shape is nearly homogeneous. The various contributions to the α band are untangled by using RREPs of vibrational modes of varying frequencies (~200 cm^{-1} to ~1600 cm^{-1}). From the pure 0,0 line shapes derived from the RREPs we determine the lifetimes of the two components of the nearly degenerate zero vibrational level of Q(r,R). The sub picosecond lifetimes obtained are consistent with the very low quantum yield of emission observed in these systems. The short lifetimes are attributable to the nonradiative coupling between the x and y components of $Q_0(r,R)$ and the iron d → d electronic states. We observe that the nonradiative lifetime of the x and y components of $Q_0(r,R)$ in ferrocytochrome-c but not b_5 are very nearly the same. The difference in lifetimes between Q_{0x} and Q_{0y} in ferrocytochrome b_5 is explainable in terms of a rhombic distortion of the electronic potential about the heme iron. We consider the use of RREPs in the determination of the relative energy spacing between porphyrin π orbitals and iron d orbitals by use of an empirical energy gap expression for radiationless transitions. This information is extremely important in correlating hemeprotein redox potentials to the orbital makeup of iron porphyrin.

W-PM-F8 A SPIN LABEL STUDY OF RESPIRATION IN MEMBRANE VESICLES OF ESCHERICHIA COLI. A.G. McAfee and C. Ho, Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pa. 15260

Spin labeled stearic acid, 2-(14-Carboxytetradecyl)-2-ethyl-4, 4-dimethyl-3-oxazolidinyloxy, has been shown to couple with carriers of the electron transport system of energized E. coli membranes [Biochemistry 13, 5210 (1974)]. The coupling site of spin label is further localized not only within the lactate oxidase chain, but in reference to the junction of the NADH, D-lactate and succinate energized carriers into a common respiratory chain. Parameters monitored include the decrease in intensity of electron paramagnetic resonance signal as well as the changes in oxygen consumption, of dehydrogenase activity, and of cytochrome b_1 kinetics while the energized state is altered by respiratory inhibitors, varied substrates, varied spin label concentrations or genetic lesions of the membrane. The results indicate spin labeled stearic acid to be a sensitive respiratory inhibitor acting before cytochrome b_1 oxidase and accessible to all three respiratory substrates. An interaction of spin label with a respiratory carrier protein or oxidized cytochrome b_1 is hypothesized. This respiratory carrier protein, either consisting of three separate proteins for each dehydrogenase chain, or one common protein accessible to all three substrates, is postulated to interact with both spin label and oxidized cytochrome b_1. (This work is supported by research grants from NSF.)

W-PM-F9 THE HEME PROTEIN P-450 FROM THE ADRENAL CORTEX: STUDIES OF THE ROLE OF THE AXIAL LIGANDS. Heinz Schleyer, David Y. Cooper[x], Otto Rosenthal[x], and Pamela Cheung[x]. Harrison Department of Surgical Research and Johnson Research Foundation, University of Pennsylvania, School of Medicine, Philadelphia, PA, 19104.

The prosthetic group of the heme protein P-450(Fe^{3+}), S = 1/2, is most likely a Fe^{3+}-protoheme IX-complex in which a thiol ligand and an aromatic N-ligand are coordinated to the metal ion in the axial 5- and 6- positions; this view is derived from spectroscopic studies of a variety of "model systems" in our as well as in many other laboratories. It appears that the thiol ligand with its pronounced electron donating properties determines the chemical and enzymatic reactivities of the heme protein and is also responsible for some of its unusual spectral properties. Optical absorption measurements and EPR spectroscopy were used to study the possible role of this thiol ligand in intramolecular charge transfer processes using P-450(Fe^{3+}) from adrenal cortex in its native form and in modified forms in which the naturally occurring "trans" ligand was replaced by pyridine- or imidazole-compounds, by nitric oxide, or by other ligands. Replacement by pyridine at neutral pH yields a new Fe^{3+} complex with distinct rather broad absorption bands accompanied by a shift of the g-tensor from (1.91, 2.24, 2.42) to (1.88, 2.25, 2.52). The analogous reaction at alkaline pH leads to the transient formation of a Fe^{2+}-complex (absorption maxima 418, 523, 556 nm) which decays with $t_{1/2}$ 5-10 min. Examples of these studies on P-450(Fe^{3+}) are described and supplemented by results of related measurements on Fe^{2+}-forms in which the thiol ligand is either maintained or removed.
These studies are supported by Grants AM-04484 (NIH) and BMS 74-01099 (NSF).

W-PM-F10 MODELS FOR ACTIVATED HEME ENZYMES: HORSE RADISH PEROXIDASE COMPOUND I AND A CYTOCHROME P-450 ANALOGUE. Gilda H. Loew, Charles Kert,* and Leonard M. Hjelmeland,* Department of Genetics, Stanford University Medical Center, Stanford, California, 94305

Semi-empirical molecular orbital calculations have been made for a model of the active site of the primary complex (Compound I) formed by Horse Radish Peroxidase with peroxides. With imidazole and an oxygen atom as axial ligands, the ground state in a S=3/2 configuration is a π cation porphyrin radical with two unpaired electrons in highly covalent (d_{π}-O_{π}) iron-oxygen orbitals. Electric field gradients and quadrupole splittings calculated for both Compound I and its reduced form (Compound II) are in good agreement with experiment. A cytochrome P-450 analogue of Compound I with axial mercaptide and atomic oxygen ligands has a similar configuration and could account for the electrophilicity of the active oxygen.

W-PM-F11 SPIN TRAPPING AND ITS APPLICATION IN THE STUDY OF LIPID PEROXIDATION AND FREE RADICAL PRODUCTION WITH LIVER MICROSOMES. A.N. Saprin* and L.H. Piette, Cancer Center of Hawaii, University of Hawaii, Honolulu, Hi. 96822

Lipid peroxidation in microsomes is studied using a spin-trapping technique. Free radical adducts of phenyltertiarybutylnitrone (PBN) are produced as detected by electron spin resonance during induced lipid peroxidation of microsomes with a system consisting of NADPH, Fe^{+2} and pyrophosphate. The adducts are identified as intermediates of the substrates added to the microsomal system and not $^{\bullet}OH$ or $HO_2^{\bullet}$ radicals. The production of the adduct parallels the NADPH dependent formation of malondialdehyde (MDA). Analyses of the ESR hyperfine splitting constants allows for identification of the adducts. Purified preparations of cytochrome P-450 mimic the results of the microsomes. The carcinogens dimethyl and diethylnitrosoamine are metabolized in this system to reactive free radicals and free NO. The results suggest that although the production of OH radicals may be the initial event leading to lipid peroxidation, the actual formation of malondialdehyde appears to take place as a result of secondary radical formation associated with the substrates added. The mechanism of lipid peroxidation and microsomal oxidation of alcohols and dimethylsulfoxide will be discussed.

W-PM-F12 "TIGHTLY-BOUND" NUCLEOTIDES ON MITOCHONDRIAL ATPase (F_1). Richard M. Leimgruber* and A. E. Senior, Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14642.

Beef-heart mitochondrial F_1 (Brooks and Senior, Biochemistry 11, 4675 (1972)) contained zero moles ATP and 1.8 moles ADP per mole F_1 after repeated precipitation with ammonium sulfate or gel filtration on Sephadex G-25 in 50mM Tris-SO_4 - 1mM EDTA, pH 8.0 (TE). Treatment with low levels of trypsin or gel filtration on Sephadex G-25 in 50mM Tris-SO_4-1mM EDTA-60mM K_2SO_4, pH 8.0 (TEK) fully depleted the F_1 of ATP and ADP; the F_1 then no longer acted as a coupling factor (Leimgruber and Senior, J. Biol. Chem., Nov. 1976). When native F_1 was reconstituted with ASU-particles plus OSCP and 1mM ATP under energized conditions the particles contained 0.89 nmoles ATP and 1.04 nmoles ADP per mg particle after repeated washing. Coupling activity as measured by reversed electron transfer (succinate$\rightarrow NAD^+$) was 27.2 nmoles NADH/mg particle/min. Trypsin-treated-F_1 reloaded 0.39 nmoles ATP and zero nmoles ADP per mg particle and had no coupling activity under identical conditions. TEK-F_1 dissolved in TEK after gel filtration and $(NH_4)_2SO_4$ precipitation had no coupling activity. TEK-F_1 dissolved in TE did reload 1.05 nmoles ATP and 1.18 nmoles ADP per mg particle and had full coupling activity. Thus reloading of tightly-bound ADP seems to be a prerequisite for coupling activity. Significant differences were observed in the number of binding *sites accessible* to ^{14}C-NBD-Cl, with native F_1 binding 12.66 ± 1.62 moles NBD-Cl/mole F_1, trypsin-treated-F_1 binding 8.87 ± 2.38 moles NBD-Cl/mole F_1, and TEK-F_1 binding 10.26 ± 2.49 moles NBD-Cl/mole F_1 (Soluble F_1 reacted with 200 µM NBD-Cl for 20 hr. at pH 8.0). Gel electrophoresis experiments have so far revealed no structural differences between native F_1 and trypsin-treated-F_1 or TEK-F_1.

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W-PM-F13 ON THE MODE OF COUPLING FACTOR–NUCLEOTIDE INTERACTIONS AND THE MECHANISM OF ATP SYNTHESIS IN OXIDATIVE AND PHOTOSYNTHETIC PHOSPHORYLATION. M.A. Tiefert, R. Adolfsen*, and E.N. Moudrianakis, Biology Department, Johns Hopkins University, Baltimore, Maryland 21218.

We have studied the interactions of adenine nucleotides with the coupling factors of electron-transport-coupled phosphorylation from spinach chloroplasts and the bacterium *Alcaligenes faecalis*. We have also examined the structure of these coupling factors during certain steps of their function. The following observations have been made in our laboratory: (A)The homogeneous coupling factors bind ADP at two types of sites with differing affinities (1,6) and have the intrinsic ability to perform a phosphoryl group transfer between two bound ADP molecules to produce a bound ATP and a bound AMP (1). (B)This transphosphorylation reaction is not due to contamination by conventional adenylate kinase (2). (C)The membrane-bound coupling factor uses AMP as a cofactor and as a first acceptor of P_i, and in an energy-dependent manner first generates bound ADP, from which it subsequently transfers the β-phosphate to substrate ADP to generate ATP without additional energy input (3). (D)Coupling factors can be isolated in several structural and functional forms; thus they are polymorphic (4). (E)During ATP synthesis and ATP hydrolysis the coupling factors operate by alternating between two end states (5). This we described as the "flip-flop" mode of their action (6). An attempt will be made to integrate our experimental observations and theoretical proposals into a simple scheme explaining the enzymology of ATP synthesis. We will also attempt to clarify certain misrepresentations of our ideas as adapted by others. Refs.: (1)*PNAS 68*, 464 (1971). (2)*J. Biol. Chem.* (Dec. 25, 1976). (3)*PNAS 68*, 2720 (1971). (4)*Biochemistry 14*, 1747 (1975). (5)*Biochemistry 12*, 2926 (1973). (6)*Arch. Biochem. Biophys. 172*, 425 (1976).

W-PM-F14 MODIFICATION OF BACTERIAL LUCIFERASE WITH 2,4-DINITROFLUOROBENZENE. W. R. Welches* and T. O. Baldwin, Biochemistry Department, University of Illinois, Urbana, Illinois 61801

Bacterial luciferase is a heterodimer (αβ) which catalyzes the oxidation of $FMNH_2$ and a long-chain aldehyde by molecular oxygen; the reaction results in light emission. Numerous lines of evidence suggest that the active center resides on the α subunit. Luciferase possesses a particularly reactive sulfhydryl group in or near the active center. This residue can be readily modified by reaction with a great variety of alkylating reagents. We have employed 2,4-dinitrofluorobenzene (FDNB) which reacts reversibly with cysteinyl, tyrosyl and histidyl residues, and irreversibly with α- and ε-amino groups, to further probe the active center of the enzyme. FDNB inactivates bacterial luciferase with an apparent second order rate constant of about 100 M^{-1} min^{-1}. The inactivation appears to result from modification of a single residue on the α subunit, and is not reversed by addition of excess thiol reagent. The enzyme is protected from inactivation by high concentrations of the product of the reaction, FMN, and to a lesser extent by long-chain aldehydes. These findings suggest the existence of an amino group (α or ε) in or near the active center of bacterial luciferase which reacts with FDNB, resulting in an inactive luciferase species. (Supported by NSF Grant PCM 76-00452).

W-PM-F15 SEPARATION AND N-TERMINAL SEQUENCE ANALYSIS OF THE SUBUNITS OF THE REACTION CENTER PROTEIN FROM R. SPHAEROIDES R-26.† D.Rosen, M.Y.Okamura, G.Feher, Physics Dept., U.C.S.D., La Jolla, Calif., L.A.Steiner, Biology Dept., M.I.T., Cambridge, Mass., J.E.Walker,* MRC Lab. of Mol. Biol., Cambridge, U.K.

The reaction center protein from Rhodopseudomonas sphaeroides R-26 contains three subunits (L, M, and H) in equimolar proportion. These have been isolated by SDS gel electrophoresis in quantities sufficient for determination of amino acid composition. Notable features were the large proportion of hydrophobic residues and the presence of half-cystine in only 2 of the subunits, L and H [Okamura, et al. and Steiner, et al., Biochem. 13, 1394-1410 (1974)]. We report here a procedure for large scale (10-100 mg) preparation of these subunits. Reaction centers were heated at 40°C for two hours in the presence of 0.5M $LiClO_4$, 10% (v/v) ethanol, 50 mM Ca^{2+}, 10 mM Tris-Cl pH 8.0, and 0.025% lauryldimethylamine oxide. All of the H subunit, together with a small fraction (∿ 10%) of the L and M subunits, precipitated. The purified LM complex in the supernatant was treated with 1% SDS and passed over a column of sepharose to which parahydroxymercuribenzoate was covalently bound. The L subunit adhered to the column and was eluted with cysteine; the M subunit was unretarded. This procedure yielded L subunits of high purity (> 98%) and H and M subunits of moderate purity (∿ 90%). Further purification of M and H is in progress. The amino terminal sequence of L, obtained in a Beckman liquid phase sequencer, was: Ala-Leu-Leu-X-Phe-Glu-Arg-Lys-Tyr-Arg-Val-Pro-Gly-Gly-Thr-Leu-Val-Gly-Gly-Asn-Leu-Phe-Asp-Phe-. In the case of the H and M subunits, only a low yield of N-terminal sequence was found.

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W-PM-F16 PHOTOREACTIVATING ENZYME: PHYSICAL AND CHEMICAL PROPERTIES OF THE ENZYME FROM ESCHERICHIA COLI. R.M. Snapka*, C.O. Fuselier*, and B.M. Sutherland, Dept. of Molecular Biology and Biochemistry, University of California, Irvine, California 92717.

The enzyme has been purified to a state of apparent homogeneity. It co-purifies with a small amount of carbohydrate which may stabilize the activity. The monosaccharide composition of this material has been determined by gas-liquid chromatography. The monomer molecular weight is estimated at 35,200 daltons by SDS-gel electrophoresis, molecular sieving in guanadinium hydrochloride, and sedimentation velocity. Higher polymers are usually evident. The enzyme displays a strong concentration-dependent self-association under non-denaturing conditions. The amino acid composition of the purified enzyme has been obtained from enzyme purified by two different methods, and is identical in each case. Several rabbits have been successfully immunized with purified enzyme. The sera inactivate the enzyme in in vitro assays, and yield one precipitin band in double diffusion experiments against the purified enzyme, but multiple bands against material from intermediate stages of the isolation.

This research was supported by Grants #CA 14005 and a Research Career Development Award (5 K04 Ca 00009) to BMS, a USPHS Traineeship (GM 07311-01) to RMS and a Teaching Assistantship to COF.

W-PM-G1 EFFECT OF CHOLESTEROL ON THE MOTIONS OF DPH IN LIPID BILAYER MEMBRANES: A DECAY OF FLUORESCENCE ANISOTROPY STUDY ON ORIENTED MEMBRANES. W.R. Veatch, Department of Pathology, Yale University School of Medicine, New Haven, Conn. 06510 and L. Stryer, Department of Structural Biology, Stanford Medical School, Stanford, Calif. 94305

The steady state fluorescence polarization of the hydrophobic membrane probe diphenylhexatriene (DPH) has been monitored by investigators seeking to estimate the effective viscosity of the interior of cell membranes. All have assumed that the motion of DPH inside the membrane is isotropic. More recently, Parola, Robbins and Blout have measured the time-resolved decay of fluorescence anisotropy of DPH bound to cells. They found that the fluorescence anisotropy did not decay to zero as it does for DPH in an isotropic solvent, rather it decayed to a non-zero plateau value. We find that in phosphatidyl choline liposomes the fluorescence anisotropy decays to zero and is fit well by a rotational correlation time of $\sim$ 8 nsec. In contrast, with the addition of cholesterol the anisotropy decays to a non-zero plateau anisotropy value which increases with increasing cholesterol mole fraction. The simplest model of a non-isotropic membrane assumes that the rotational correlation time of DPH oriented parallel to the lipid acyl chains is not equal to that of DPH oriented perpendicular. To determine the degree of orientation of DPH and to assign the fast and immobile components of the decay of fluorescence anisotropy, we have carried out the first decay of anisotropy measurements on oriented multilayers. The initial anisotropy values for the various membrane orientations showed that the DPH molecules were not strongly oriented even in the presence of 33 mole % cholesterol. However the plateau anisotropies showed orientation parallel to the acyl chains. We conclude that the addition of cholesterol does not alter the rotational motion of DPH molecules oriented perpendicular to the acyl chains, but highly immobilizes those oriented parallel to the acyl chains.

W-PM-G2 SIMULTANEOUS OBSERVATION OF INTRA- AND INTER-CHAIN ORDER IN PHOSPHOLIPID DISPERSIONS BY RAMAN SPECTROSCOPY. Bruce P. Gaber and Warner L. Peticolas, Department of Chemistry, University of Oregon, Eugene, Ore. 97403.

Raman spectra of phospholipids contain two regions known to be particularly sensitive to the conformation of acyl chains: the skeletal optical modes (SOM) (1050-1150 cm^{-1}) and the methylene stretching modes at 2850 cm^{-1} and 2890 cm^{-1}. It is our observation that these spectral regions each yield unique, but complementary conformational information. The relative intensity and qualitative behavior of the skeletal optical mode near 1133 cm^{-1} provides quantitative insight into intra-chain conformation. Assuming that the intensity of this C-C stretching vibration is the sum of intensities from individual all-trans segments, a formula for the Raman intensity has been obtained in terms of the probability of occurrence of a trans bond, the number of bonds in an all-trans sequence within a molecule of N bonds, and the number of times the nth segment recurs. Application of this formalism permits the estimation of the total number of trans bonds in sequences of 3 or more bonds. For dipalmitoyl lecithin at T_M, this number is $\sim$8 bonds. Inter-chain order is estimated by the methylene stretching intensities. Experiments in which a hydrocarbon chain is isolated vibrationally from its neighbors demonstrate that the total relative intensity ($I_{tot} = I_{2890}/I_{2850}$) of a highly ordered crystalline hydrocarbon lattice can be broken into three parts: I_{liquid} = 0.7, the residual intensity in the liquid; $I_{rotamer}$, the difference between the intensity of a liquid and that of an isolated all-trans chain; and $I_{lateral}$, that due to vibrational coupling between the adjacent chains in the hydrocarbon crystal. The two contributions to I_{tot} may be differentiated by an associated frequency change. (Supported in part by NSF and NIH grants to W.L.P.; Dr. Gaber holds Nat. Cancer Fellowship, No. CA 5488-01).

W-PM-G3 DYNAMICS OF THE PLASMA MEMBRANE OF RABBIT PLATELETS. Elliott Berlin, Lipid Nutrition Laboratory, Nutrition Institute, Agriculture Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705

The fluidity of the hydrocarbon region of the rabbit platelet plasma membrane was determined by measuring the anisotropy of fluorescence from the probe 1,6-diphenyl-1,3,5-hexatriene. Microviscosity, η, and fusion activation energy, ΔE, in the lipid core of membrane isolates and in liposomes prepared from lipid extracts of the membranes were determined between 0 and 60°C. Both systems exhibited similar microviscosities, which at 37°C were 3.9±.1 poise for the membranes and 4.5±2.1 poise for the liposomes, however, they differed in fusion activation energy. The membranes were characterized by ΔE= 10.8±1.9 kcal/mole while the liposome hydrocarbon phase was characterized by ΔE= 5.8±1.1 kcal/mole, suggesting a membrane stabilization role for the membrane proteins. A much lower value of ΔE= 2.0 kcal/mole was obtained with intact platelets for the hydrocarbon phase existing between 0 and 39°C, while the microviscosity at 37°C was 3.7 poise. These results may reflect unique membrane dynamics in the intact cell or possible entry of the probe into membranes in the cell interior through the canalicular system.

W-PM-G4 MYELIN PARACRYSTALLINITY. A. Hybl, Department of Biophysics, University of Maryland School of Medicine, Baltimore, MD. 21201

An analysis# of thickness variances from high resolution electron microscopic cross sections of myelin sheath have shown that the lamellar stacking is paracrystalline. Paracrystallinity means that myelin can no longer be considered a simple one-dimensional crystalline material. The discovery that myelin scatters X-rays gave birth to a simple crystalline model. A long habit of not thinking that the crystalline model is wrong has given it the superficial appearance of being right.

The X-ray diffraction data from myelin is re-examined. Friede and Samorajski (1967) have shown that the number of lamellae per fiber in various nerve bundles range roughly between 5 and 100 (see insert). Size distribution influences the peak profile observed in X-ray studies.

The X-ray reflection intensity decline and profile broadening are consistent with a paracrystalline model.

Number of Lamellae
Composite Histogram from the
Sciatic Nerves of Mice

*Supported by NSF grant GB 43447 and NIH grant CA 16773.

#Hybl, A. (1976) Mol. Cryst. Liq. Cryst., in press.
Friede, R. L. & Samorajski, T. (1967) J. Comp. Neur. 130: 223-232.

W-PM-G5 SWELLING PROPERTY OF MEMBRANES, N. S. Murthy and C. R. Worthington, Departments of Biological Sciences and Physics, Carnegie-Mellon University, Pittsburgh, Pa. 15213.

In recent x-ray work on multilayered membrane-type systems which contain fluid layers, it has been demonstrated that the membrane structure remains unchanged during swelling. The net result of swelling, for example, in the case of nerve myelin, is an increase in the widths of the fluid layers between adjacent membranes. It has been noted previously that swelling (of nerve myelin) takes place abruptly and moreover different repeat distances are obtained in different experiments even when using the same immersion fluid. This has always been puzzling, but we now note that certain repeat periods tend to occur more often than one would expect if the swelling was a continuous process. We have analyzed swelling data from sciatic and optic nerves and from sarcoplasmic reticulum membranes (x-ray data obtained in our laboratory) and we find that the increases in x-ray period can be accounted for in terms of nw where n is an integer and w is a constant. It is suggested that w refers to the width of a monolayer of water molecules. Our analysis and statistical tests of this hypothesis will be presented.

W-PM-G6 EFFECT OF THE HYDROPHOBIC PEPTIDE T(IS) FROM MN-GLYCOPROTEIN ON NA^+ EFFLUX AND DIFFERENTIAL SCANNING CALORIMETRY OF PHOSPHOLIPID BILAYER VESICLES. A.Y. Romans, T.M. Allen, and J.P. Segrest, Pathology Department, University of Alabama Medical Center, Birmingham, Alabama 35294

Increasing concentrations of T(is) were added to phosphatidylcholine vesicles and the rate of efflux of $^{22}Na^+$ was examined. Below the critical multimer concentration (CMC) of T(is) in the bilayer, Na^+ leaks out at a constant low rate. Above the CMC the rate of efflux is biphasic, exhibiting both an initial fast and a subsequent slow component. Examination of the rate of efflux as a function of T(is) concentration suggests that the low rate of efflux below the CMC and the slow component of the biphasic curve both result from association of T(is) monomers with the lipid bilayer while the fast component is due to the formation of T(is) multimers within the bilayer. Differential scanning calorimetry (DSC) of T(is) associated with lipid bilayers shows that there is a large decrease in the enthalpy of transition ΔH_t as more T(is) is incorporated into the bilayer with a change in slope at the CMC. In addition there is small but significant decrease in the temperature of the phase transition Tc. Work is presently underway to distinguish between several models which can explain both the ion flux and DSC data.

W-PM-G7 CIRCULAR DICHROISM STUDIES ON THE STRUCTURE OF fd COAT PROTEIN IN ARTIFICIAL MEMBRANES. Robert Williams,* Department of Chemistry, Boise State College, Boise, Idaho and A. K. Dunker, Program in Biochemistry and Biophysics, Washington State University, Pullman, WA. 99164.

Circular dichroism studies on fd coat protein incorporated into lipid vesicles show that at least three different membrane-associated protein conformations can exist: (1) vesicles prepared by sonication as described by Nozaki et al., 1976 give protein with $[\theta]_{208}$=-14.0 deg m^2 $mole^{-1}$; (2) vesicles prepared by sonication but using phenol extracted protein give protein with $[\theta]_{208}$=-2.71 deg m^2 $mole^{-1}$; (3) vesicles prepared by the cholate dilution method (Racker et al., 1975) as described by Wickner, 1976, give protein with $[\theta]_{208}$=+4.36 deg m^2 $mole^{-1}$. Furthermore, additional experiments demonstrate that the sonication method incorporates the protein in the same conformation as that exhibited by the protein in comparable solutions without lipid. These results cast doubt on the recent proposal suggesting that large conformational changes occur in the filamentous virus coat protein during virus assembly. Furthermore, these results also suggest that equilibrium thermodynamics may not provide a valid criterion for judging the structural possibilities of protein in biological membranes.

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Nozaki, Y., Chamberlain, B. K., Webster, R. E., and Tanford, C. (1976) Nature 259, 335-337.

R. W. was supported by an NSF undergraduate research participation grant, SMI-76-03088.

W-PM-G8 DEUTERIUM NMR OF MEMBRANES: THE ROLE OF CHOLESTEROL AND THE CO-EXISTENCE OF GEL AND LIQUID CRYSTAL LIPIDS. Ian C.P. Smith, G.W. Stockton*, K.G. Johnson*, K.W. Butler, J.H. Davis*+, M. Bloom*+ and A.C. Oehlschlager*. Div. Biol. Sci., National Research Council, Ottawa, Canada K1A 0R6.

Specifically-deuterated phospholipids, fatty acids and cholesterol have been used to study the interaction between cholesterol and phospholipids in model systems and in the microorganism Acholeplasma laidlawii. Parallel ESR spin label experiments were done in each case for comparison. The condensing effect of cholesterol is strongest near the glycerol region of the phospholipids and decreases rapidly towards the center of the bilayer. In egg lecithin the ESR data yield a stronger condensing effect for position-12 of the fatty acyl chains than for position-5, a contradiction presumbably due to the perturbing influence of the nitroxide moiety. The isopropyl methyl groups of cholesterol have low but different degrees of order at low cholesterol concentrations; their degrees of order increase with increasing concentration becoming equal to one another at ca. 30 mole % cholesterol. Conversely, the steroid nucleus is highly ordered. No evidence is obtained for specific complexes of cholesterol and egg lecithin below 30 mole % cholesterol. Coexistence of gel and liquid crystalline phases of the membrane lipids of A. laidlawii is observed over the temperature range 15-60°. The gel phase can comprise 60% of the lipid in viable A. laidlawii.

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W-PM-G9 Characterization of Neisseria gonorrhoeae by Spin Label Electron Spin Resonance. W.J. Newhall, F.W. Kleinhans*, R.S. Rosenthal*, M.K. Stine*, W.D. Sawyer* and R.A. Haak, Med. Biophys., Physics (IUPUI), and Microbiology, Ind. U. Sch. of Med., Indpls., IN. 46202.

Spin labels which partition in the cytoplasm (PCA and tempone) and cell envelope (n-doxyl stearic acid) were used to characterize different clinical isolates and laboratory strains of the bacterial pathogen, N. gonorrhoeae. Four colonial types of strain 2686 were also compared. Suspensions of intact cells measured at 25°C were labeled for 1 min. Cells measured at 37°C were preincubated for 30 min with 4 mM NEM and labeled for 10 min. The order parameters thus obtained were consistent with published data for fractionated cells of other bacterial species and model membranes. The concentrations of spin label (< 3 x 10^6 molecules/cell) did not affect colony forming ability and did not cause detectable changes in membrane fluidity. Preliminary spin label localization measurements indicated that for short labeling periods the ESR signal of the lipid labels originated primarily from the outer membrane of the cell envelope. After longer labeling periods, both reduction of the spin label and Ni^{2+} broadening studies suggested that some label had reached the cytoplasmic membrane. Measurement of τ_c and S indicated a) the different clinical isolates and laboratory strains of N. gonorrhoeae were similar (S ≃ 0.69), b) colonial types T1, T2 and T3 are also similar, c) T4 cells had a slightly lower order parameter at 25°C (S ≃ 0.68), d) a flexibility gradient was detected similar to those found in other membrane systems, e) the cytoplasmic microviscosity, as measured by low molecular weight spin labels, was approximately 4x that of distilled water. This method is now being applied to the study of the cell envelope characteristics involved in pathogenicity and antibiotic resistance.

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W-PM-G10 THE LATERAL MOTION AND VALENCE OF Fc RECEPTORS ON RAT PERITONEAL MAST CELLS. J. Schlessinger‡ H. Metzger‡ W. W. Webb and E. L. Elson‡ Cornell University, Ithaca, N.Y. 14853; NIAMDD, National Institutes of Health, Bethesda, Md. 20014 (H.M.)

Mast cells and basophils bind monomeric IgE antibodies reversibly but tightly via specific Fc receptors on the surface. Limited bridging of bound IgE molecules by multivalent antigens or anti-IgE antibodies releases stored granules, liberating histamine and other mediators of the allergic response. We have measured the lateral mobility of dispersed rhodamine IgE (R-IgE) or fluorescein IgE (F-IgE) on rat peritoneal mast cells and the effects on mobility of conditions that induce or inhibit degranulation. The mobility was measured by Fluorescence Photobleaching Recovery (FPR) [Schlessinger et al., PNAS 73, 2409 (1976); Axelrod et al., Biophys. J. 16, 1055 (1976)]. We find that most IgE-Fc receptor complexes are mobile with $D = (2.1\pm0.1)\times10^{-10}cm^2/sec$ but 20-50% are immobile on our experimental time scale. Limited cross-linking by antifluorescein that triggers degranulation does not affect the apparent mobility of receptors. Extensive aggregation inhibitory of degranulation, however, abolishes the mobility. Cytochalasin B reduces the lateral mobility of the complexes; colchicine has little detectable effect. We have also tested the valence of the Fc receptor for IgE. Mast cells were labeled with equal amounts of F-IgE and R-IgE and the two fluorophores were separately excited by 482nm and 568.2nm lines of a krypton ion laser. The labeled cells were then tagged with antifluorescein antibodies from horse and antihorse antibodies, respectively. This treatment immobilized all of the F-IgE-receptor complexes and none of the R-IgE receptor complexes, indicating that the Fc receptor is monovalent for the IgE and there is no bridging between the Fc receptors [Mendoza and Metzger, Nature (1976) in press; Schlessinger et al., Nature (1976) in press.

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W-PM-G11 NONEXCHANGEABLE ^{3}H-CHOLESTEROL IN METABOLICALLY LABELLED INTACT MOUSE ERYTHROCYTES. M.J. Poznansky, and Y. Lange. Dept. of Physiology, University of Alberta, Edmonton, Alberta and Biophysics Division, Boston University School of Medicine, Boston, Mass.

Mouse erythrocytes were labelled metabolically with ^{3}H-cholesterol by induction of reticulocytosis followed by repeated injections of the cholesterol precursor ^{3}H-labelled mevalonic acid. Over 95% of the ^{3}H associated with the intact washed red cells was incorporated in cholesterol. To determine whether the red cell cholesterol existed in one or more identifiable pools, .5cc of labelled red cells (.38 μm Phospholipid/cc) in phosphate-buffered saline (pH 7.4) and 5 cc of sonicated unilamellar vesicles (2.0 μm Phospholipid/cc) containing a 0.9 mole ratio of cholesterol to egg lecithin and trace amounts of ^{14}C-labelled cholesterol-oleate as non-exchangeable marker, were co-incubated at 37°C. Red cells and lipid vesicles were separated by centrifugation and the exchange of ^{3}H-cholesterol from the red cells to the vesicles was determined as a function of time. Depending on the degree of reticulocytosis and the amount of ^{3}H-mevalonate injected, up to 32% of the labelled cholesterol is nonexchangeable in 24 h. At the concentration of red cells and vesicles indicated, and in the absence of any net movement of cholesterol, the exchange is described by a single exponential with a half time of 1.5 h. When the ^{3}H-cholesterol labelled red cells are osmotically lysed and an exchange experiment performed with the leaky ghosts, over 90% of the labelled cholesterol is available for exchange with the vesicle cholesterol. Because ^{3}H-cholesterol in the red cell may be derived from either plasma cholesterol or from de-novo synthesis during reticulocytosis, no statement of absolute pool size may be made. It appears that a nonexchangeable pool of approximately 30% exists in intact mouse erythrocytes and that this pool becomes available for exchange upon the production of leaky ghosts.

W-PM-G12 THE STRUCTURAL REQUIREMENTS OF LECITHIN (PC) FOR ACTIVATION OF D-β-HYDROXYBUTYRATE DEHYDROGENASE (BDH). Y.A. Isaacson*, P.W. Deroo*, A.F. Rosenthal*†, and R. Bittman*, Chemistry Dept., Queens College, City Univ., Flushing, N.Y., 11367, †Dept. of Laboratories, Long Island Jewish Hillside Medical Center, New Hyde Park, New York, N.Y., 11040, and J.O. McIntyre*, H.-G. Bock*, P. Gazzotti*, and Sidney Fleischer, Dept. of Molecular Biology, Vanderbilt Univ., Nashville, Tennessee 37235

BDH, a lipid-requiring enzyme purified to homogeneity from bovine heart mitochondria, has an absolute and specific requirement for PC. To determine which portion of the lecithin molecule is essential for BDH activation, a number of analogues were synthesized and tested after co-dispersing with phosphatidylethanolamine (PE) and diphosphatidylglycerol, the other major components of mitochondrial phospholipid (MPL). We find: (1) The hydrophobic portion is essential but its precise structure is unimportant since: a) the D and L isomers work equally well, and b) a branched alkyl chain analogue without the glycerol moiety works as well as MPL. (2) The phospho-glycerol ester oxygen is not essential since phosphono analogues (oxygen missing or replaced by a CH_2 group) activate the enzyme. However, phosphinate analogues (both oxygens removed) do not activate. (3) The quaternary ammonium moiety of choline is essential. No activation was obtained with either PE or N,N-dimethyl or N,N,N-triethyl ethanolamine analogues but the N-ethyl-N,N-dimethylethanolamine analogue activates BDH. (4) The distance between the two charged groups of the phosphorylcholine moiety is not critical. When this distance is extended by either one or two CH_2 groups, full activity remains. However, if the ethylene is replaced with an isopropyl group, there is no activation. (5) Sphingomyelin does not satisfy the PC requirement. Thus, the structure of the polar portion, the zwitterionic character and steric factors have profound influence. [Supported in part by LIJHMC 3-265(to AFR), NIH AM14632(to SF) and HL16660(to RB)]

W-PM-G13 EFFECT OF THE LIPID ENVIRONMENT ON PROTEIN MOTION AND ENZYMATIC ACTIVITY OF THE SARCOPLASMIC RETICULUM CALCIUM ATPASE. David D. Thomas and Cecilia Hidalgo, Dept. of Muscle Research, Boston Biomedical Res. Inst., 20 Staniford St., Boston, MA 02114

Replacement of 90-95% of the endogenous phospholipids of the Ca^{2+}-ATPase enzyme of fragmented SR by dipalmitoyl lecithin (DPL) yields a partially purified enzyme preparation (DPL-E) which at temperatures below 29° has a strongly inhibited ATPase activity. The formation of phosphoenzyme (E~P) proceeds normally; the inhibition takes place at the level of E~P decomposition and is completely reversed at temperatures above 29° or by Triton X-100, suggesting a requirement for a fluid lipid environment in the late steps of the ATPase reaction. EPR experiments with stearic acid spin labels incorporated into DPL-E indicate that at low temperatures the lipid phase is strongly immobilized. Raising the temperature or adding Triton X-100 results in a significant increase in the mobility of the lipids. Labeling the protein moiety of DPL-E with a maleimide spin label yields conventional (first derivative) spectra that indicate a strong immobilization of the label (rotational correlation time $> 10^{-7}$ s); only very small changes are observed on raising the temperature from 0° to 40° or adding Triton X-100. Further analysis by saturation transfer EPR, a method sensitive to much slower motions than the conventional EPR method, clearly indicates that at 4° the protein in DPL-E is very strongly immobilized (correlation time $\geq 10^{-3}$ s), and that a significant increase in the rotational motion of the protein occurs when Triton X-100 is added or the temperature is raised. This increase in protein motion correlates with the increase in enzyme activity that occurs under the same conditions in unlabeled preparations. We have performed similar experiments with ATPase preparations containing variable amounts of SR lipids in a further effort to correlate the lipid environment, the motion of the protein, and the enzymatic activity in the Ca^{2+}-ATPase complex. (Supported by grants from NIH, MDAA, AHA and NSF; D.D.T. is a Research Fellow of MDAA.)

W-PM-G14 DISPOSITION OF PROTEINS AND LIPIDS IN THE SARCOPLASMIC RETICULUM MEMBRANE. Cecilia Hidalgo and Noriaki Ikemoto, Department of Muscle Research, Boston Biomedical Research Institute, 20 Staniford St., Boston, MA 02114

Sarcoplasmic reticulum (SR) membrane vesicles have been reacted with a non-penetrating water-soluble complex of fluorescamine with cycloheptamylose (CFC, Nakaya et al., BBRC 67, 760, 1975). The reaction of CFC with primary amino groups, measured by the increase in fluorescence intensity, is complete after 20 min of incubation at 37° without affecting the permeability of the SR vesicles as evidenced by their impermeability to [^{14}C]-inulin. The specific incorporation of CFC into each membrane component has been determined after solubilization of the labeled membrane with SDS, followed by fractionation and purification of the main protein components on Biogel P-150 columns equilibrated with SDS. The highest degree of label incorporation was found in a glycoprotein factor of 30,000 M_r. The specific incorporations into the ATPase peptide and calsequestrin were 64% and 22%, respectively, of that found in the glycoprotein. Incorporation of the label into calsequestrin *in situ* was only 10% of that observed with purified calsequestrin in solution. These results suggest that most calsequestrin molecules are either buried in the phospholipid bilayer or exposed at the inner surface. The label incorporation into the amino-containing phospholipids of SR reveals that most of the phosphatidyl ethanolamine (PE) is available to external labeling with CFC, while only a fraction of the phosphatidyl serine (PS) is available; this indicates that most of the PE is located at the exterior side of the membrane and that PS is enriched at the inner side. (Supported by grants from NIH, MDAA, and AHA.)

W-PM-G15 STUDIES OF INTERACTIONS BETWEEN LIPIDS AND A MEMBRANE-BOUND ENZYME. L.W.-M. Fung, E.A. Pratt,* J.A. Flowers,* and C. Ho, Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pa. 15260

A cytoplasmic membrane-bound enzyme, D-lactate dehydrogenase, of *Escherichia coli* W3110trpA33, has been solubilized by detergents and purified 2,500 fold. The enzyme, which has a molecular weight of approximately 70,000, is active in the respiration chain and in the transport of various amino acids and sugars into *E. coli* cells. The purified, detergent-free enzyme has been used for the study of lipid-protein interactions. Enzymatic studies indicate that the oxidation of D-lactate by this enzyme preparation is enhanced by amphipathic compounds like non-ionic detergents and phospholipids. Magnetic resonance techniques, such as ^{1}H and ^{31}P nuclear magnetic resonance spectroscopy and electron paramagnetic resonance spectroscopy employing spin label probes, have been used to monitor the structure and environment of the lipids in the presence and absence of purified D-lactate dehydrogenase fraction. The relationship between the enzymatic activities and the spectral properties observed under different conditions will be discussed. (This work is supported by research grants from NSF and NIH.)

W-PM-G16 DIETARY MANIPULATION OF CHOLESTERYL ESTER MELTING TEMPERATURES IN RABBIT LOW DENSITY LIPOPROTEINS. G. Hillman, S. Mabrey, F. Pitlick* and D. Engelman, Molecular Biophysics and Biochemistry Dept., Yale University, New Haven, CT. 06511

At any temperature, the physical state of cholesteryl esters (CE) is primarily a function of their fatty acid (fa) composition. In attempt to manipulate the melting points (mp) of CE's associated with low density lipoproteins (LDL), male New Zealand rabbits were fed a 0.5% cholesterol diet enriched with either unsaturated fa's (6% safflower (SO)) or saturated fa's (6% hydrogenated coconut oil (CO)) for 1 month. Serum LDL's were isolated by density centrifugation and studied by differential scanning calorimetry. SO-enriched LDL's underwent a sharp reversible phase transition at 27°C and CO-enriched LDL's underwent sharp reversible transitions at 43° and 49°C. No other reversible transitions were detected from 10° to 90°C. That the transition is reproducible after heating to 90° suggests that it is a lipid transition. To show this, LDL's were examined by x-ray diffraction (XRD) and their CE states identified. SO-enriched LDL's exhibited the CE smectic diffraction pattern at 17°C and the CE cholesteric or liquid diffraction pattern at 28°C. Similarly the CO-enriched LDL's were in the smectic state at 35°C. Since no smectic pattern was observed at 46°C it is probable that the two observed transitions correspond to the smectic to cholesteric melt (43°) and the cholesteric to liquid melt (49°C). The latter transition is difficult to detect by XRD. Thus, the physical state of cholesteryl esters at body temperature may be altered by dietary manipulation.
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W-PM-H1 SUB-PICOSECOND AND PICOSECOND DYNAMICS OF THE CONVERSION OF LIGHT ENERGY INTO CHEMICAL ENERGY BY BACTERIORHODOPSIN. A. Lewis, M.A. Marcus‡ E.P. Ippen‡ C.V. Shank‡ M.D. Hirsch* and H. Mahr‡ Cornell University, Ithaca, N.Y. 14853; and Bell Telephone Laboratories, Holmdel, N.J. 07733 (E.P.I. and C.V.S.)

We have used sub-picosecond absorption spectroscopy of transient species and picosecond emission lifetime measurements ot study the primary photochemical event in bacteriorhodopsin. Using sub-picosecond pulses from a passively mode-locked cw dye laser we have detected an increase in absorbance at 615nm in <1 psec. We have also been able to measure with an actively mode-locked cw tunable dye laser the emission lifetime of bacteriorhodopsin to be 15±3 psec at physiological temperatures. Based on the experimental conditions we believe that this is the lifetime of the emission without complications due to annihilation phenomena. This lifetime together with the measured emission quantum efficiency indicates that the emission originates from a state other than the allowed excited singlet. The transient absorption at 615nm suggests that batho bacteriorhodopsin is formed in <1 psec. The combination of the emission lifetime and the transient absorption measurements indicate that light absorption into the allowed state effectively directs the excited molecule into the batho intermediate in which part of the photon energy is stored [Lewis, Biophys. J. 15, 174a (1976)]. In moving along the excited state reaction coordinate some of the molecules vibrationally relax into an intermediate low energy state of bacteriorhodopsin from which the 15±3 psec emission finally emanates. Our experiments which suggest that the batho state is generated in <10 vibrational periods of the molecule indicates that batho production efficiently competes with vibrational relaxation accounting for the low quantum efficiency of emission. The data offers credibility to a model of photon induced electron movements for the primary photochemical event.

W-PM-H2 FLOW RESONANCE RAMAN STUDIES OF THE PURPLE MEMBRANE.† B. Aton*, A. Doukas*, R.H. Callender, B. Becker, T. Ebrey, Physics Department, City College of N. Y. and Department of Physiology and Biophysics, University of Ill.

We have measured the individual resonance Raman spectra of the BR(570) and M(412) forms of light adapted Halobacterium halobium using the newly developed flow technique described previously (Callender et al., Biochem. 15, 1621, 1976). In agreement with previous work, the Raman data indicate that the retinal is joined to the purple membrane protein via a protonated Schiff base in the case of the BR(570) form and an unprotonated Schiff base for the M(412) form. The spectra of the two forms are markedly different from each other and different from spectra of solutions of protonated and unprotonated isomers of retinal-n-butylamine. This latter result is surprising in view of chemical extraction experiments performed by other workers of the purple membrane chromophore which yield all-trans retinal for the BR(570) form and a mixture of all-trans and 13-cis retinal for M(412). The Raman data appear to indicate that the retinal of these two forms in the protein is in an intermediate configuration not found in solution.

†Supported by C.U.N.Y. Faculty Award Program, Research Corporation, N.S.F. (BMS 75-03020), and U.S. Public Health Service (EYO - 1323).

W-PM-H3 THE QUANTUM EFFICIENCY FOR THE PHOTOCHEMICAL CONVERSION OF THE PURPLE MEMBRANE PROTEIN. B. Becher and T. G. Ebrey, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801.

Using the photobleaching of bovine rhodopsin as a photon counter, we have determined the quantum efficiency for the formation of M412, an intermediate product in the photoconversion of the purple membrane protein of Halobacterium halobium. We found $\gamma_{PM568 \rightarrow M412}$ = 0.30 ± 0.03 at -40°C. This photochemical reaction was photoreversible to the original pigment and the ratio of the quantum efficiencies $\gamma_{PM568 \rightarrow M412}/\gamma_{M412 \rightarrow PM568}$ was 0.39 ± 0.02. No change was seen in either value when exciton interaction between chromophores was eliminated. The sum of $\gamma_{PM568 \rightarrow M412}$ plus $\gamma_{M412 \rightarrow PM568}$ was 1.07 ± 0.10, approximately 1, suggesting that the pigment and its primary photoproduct share a common excited state [discussed in Rosenfeld et al. (1976) Pure Appl. Chem., in press].

W-PM-H4 EXCITON INTERACTION AND CHROMOPHORE ORIENTATION IN THE PURPLE MEMBRANE. B. Mao, B. Becher, P. Kilbride, and T.G. Ebrey, University of Illinois, Urbana, Illinois 61801; and B. Honig, Hebrew University, Jerusalem, Israel.

The pigment molecules of the purple membrane of H. halobium have been shown to be arranged in clusters of three having P_3 symmetry about an axis perpendicular to the plane of the membrane. Exciton interaction exists between the retinal chromophores, and as a consequence, there is a splitting of the three originally degenerate excited-state energy levels of the individual chromophores into two new levels separated by $3V_{g,e}$, where $V_{g,e}$ is the interaction energy between two chromophore transition moments. Furthermore, three experimental exciton-band properties (the energy level splitting, the ratio of the intensities of the exciton absorption bands, and the rotational strengths of the exciton CD bands) are related through three equations to three coordinates specifying the relative positions of the chromophores. The three coordinates are the distance between two chromophores and the two angles of orientation of the transition moments relative to membrane and to the symmetry axis. The exciton contribution to the absorption and CD spectra can be obtained from accurately measured spectra of interacting chromophores (trimers) as well as noninteracting chromophores (monomers) which were made by partial and complete regeneration of bleached membrane [Becher and Ebrey, Biochem. Biophys. Res. Comm. 69, 1 (1976)]. Computerized curve fitting of these spectra were performed to obtain the three exciton-band properties which were then used in the appropriate equations to solve for the relative orientation of the chromophores. We found that all three transition moments lie ca. 19° out of the plane of the membrane, are pointed almost exactly toward the P_3 symmetry axis, and that the center-to-center distance between the transitions are about 12 Å. An energy level diagram relating the trimer levels to that of the monomer was also obtained.

W-PM-H5 SYNTHETIC PIGMENT ANALOGUES OF THE PURPLE MEMBRANE PROTEIN. (Intro. by R. Gennis), Fumio Tokunaga and Thomas G. Ebrey, Department of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801; and Rosalie Crouch, Departments of Ophthalmology and Biochemistry, Medical University of South Carolina, Charleston, South Carolina 29401.

The binding of retinal analogues to bleached purple membrane protein to form synthetic pigments was studied. All-trans $retinal_2$ combined with the bleached purple membrane to form a blue-colored membrane. The blue membrane has a light-adapted form (λ_{max}; 603 nm at 6°C) and a dark-adapted one (λ_{max}; 594 nm at 6°C). The extinction coefficients of the light- and dark-adapted forms were 0.90 and 0.80 compared to that of the light-adapted purple (naturally occurring $retinal_1$) membrane. The blue membrane has a biphasic exciton contribution to the CD spectrum of similar shape and magnitude to that seen in the purple membrane, but the magnitude of the intrinsic CD band was larger than the corresponding CD band of purple membrane. The rate of dark adaptation of blue membrane was much faster (~6 times at 25°C) than that of purple membrane. The activation energy of dark adaptation of blue membrane in 50% sucrose was 15 Kcal/mole, while that of purple membrane regenerated with all-trans retinal was 31 Kcal/mole. When blue membrane fragments were combined with soybean phospholipids and vesicles formed, light-induced pH changes could be observed similar to those reported by others with the native purple membrane. Thus, the blue membrane appears to act as a light-driven proton pump. All-trans and 13-cis retinal analogues having one extra double bond in their side chain also combined with bleached purple membrane to form pigments. Unlike the above pigments, we couldn't observe light-induced pH changes when the long-chain synthetic pigments were incorporated into vesicles.

W-PM-H6 COMPARISON OF BACTERIAL AND ANIMAL RHODOPSIN: A HYDROGEN EXCHANGE STUDY. S.W. Englander and J.J. Englander,* Dept. of Biochem. and Biophys., Univ. of Penna. Phila. 19174.

Though rhodopsin is deeply embedded in membrane, hydrogen exchange studies show it to have 70% of its peptide protons exposed to water. This contrasts with other aqueous and membrane-bound proteins which have only about 30% exposure. These and other results suggested that rhodopsin has perhaps half of its chain at the surface of a wide, water-filled channel largely penetrating the bilayer (Downer and Englander, Nature 254, 625, 1975). Subsequently Unwin & Henderson (Nature 257, 28, 1975) showed that membrane-embedded bacteriorhodopsin is arranged in a circle of three molecules which penetrate the bilayer and surround an open space 20 Å in diameter. However this space is filled not with water but with lipid and the "channel" walls are α-helical, so that bacteriorhodopsin should be as extensively hydrogen bonded as the usual protein. Hydrogen exchange results now show that bacteriorhodopsin, in agreement with the Unwin-Henderson model and unlike animal rhodopsin, has about 75% of its peptides internally H-bonded. However, in spite of the gross discrepancy in fractional H-bonding, it appears that the structured regions of these two proteins are not very different in size; animal rhodopsin, considerably the larger protein, has its extra chain length and more invested in the exposed peptide region. This and other available results are consistent with a structural (evolutionary?) relatedness for these proteins. All these observations suggest that both these proteins consist of several helix-containing monomers surrounding a large non-proteinaceous space, but that their different functions are served by maintaining an impermeable lipid core in bacteriorhodopsin and an aqueous channel in animal rhodopsin. (Supported by NIH grant AM 11295).

W-PM-H7 KINETIC RESONANCE RAMAN SPECTROSCOPY, LOW TEMPERATURE SPECTROSCOPY AND PHYSIOLOGICAL INVESTIGATIONS OF BACTERIORHODOPSIN AND BACTERIORHODOPSIN ANALOGS. A. Lewis, J.P. Spoonhower, M.A. Marcus* and A.T. Lemley*, Cornell University, Ithaca, N.Y. 14853

We have developed a dual beam flow technique and have detected kinetically at room temperature (302K) the resonance Raman spectrum of M_{412}. This spectrum is clearly modeled by an all-trans unprotonated Schiff base of retinal. We have also detected the spectrum of bacteriorhodopsin (bR_{570}) at 302K and 77K. This data can be modeled in terms of an all-trans protonated Schiff base in agreement with the biochemical evidence and can account for the color of bacteriorhodopsin in terms of excited state charge stabilization. However the modeling is not unique. To help elucidate the spectrum of bR_{570} we have investigated analogs of bacteriorhodopsin in which the chromophore is replaced by 3-dehydroretinal or 9 desmethyl retinal and/or the use of fully deuterated bacteriorhodopsin. Proton pumping and light-dark adaptation has been demonstrated for all of these analogs. In addition the absorption spectral shifts observed follow the same pattern observed for mammalian rhodopsin analogs. Low temperature spectroscopy has allowed us to identify the bands due to bathobacteriorhodopsin and has permitted us to estimate the resonance Raman spectrum of pure bathobacteriorhodopsin at 77K by varying environmental conditions to alter the batho concentration in the photostationary mixture. This allows us to account for the effect of temperature on the bR_{570} spectrum. None of the models, such as double or single bond isomerization H^+ movement, etc., that have been suggested for the primary photochemical event are successful in explaining our data. However electron movement appears to be a possibility. This suggestion is supported by sub-picosecond and picosecond dynamics of the primary events reported elsewhere in these abstracts.

W-PM-H8 RESONANCE RAMAN SPECTROSCOPY OF PROTONATED SCHIFF BASES OF RETINALS: MODELS FOR THE RHODOPSIN CHROMOPHORE. T.B. Freedman*, R. Mathies† and L. Stryer§, Yale University, New Haven, CT 06520. Present addresses: †, Chemistry Department, University of California, Berkeley, CA 94720; §, Department of Structural Biology, Stanford Medical School, Stanford, CA 94305.

We have obtained the resonance Raman spectra of solutions of all-*trans*, 11-*cis*, 9-*cis* and 13-*cis* retinals and their unprotonated and protonated Schiff base derivatives. The aim is to interpret the resonance Raman spectra of rhodopsin and its photolytic intermediates and thereby elucidate the initial conformational changes in visual excitation. A recently developed rapid flow technique which insures a continuous large fraction of unphotolyzed molecules in the illuminated volume, has enabled us to observe resonance Raman scattering from these photolabile species. The similarities in frequency and intensity between the resonance Raman lines of rhodopsin (971, 1000, 1018, 1216, 1240, 1270, 1545 and 1660 cm^{-1}) and those of the 11-*cis* protonated Schiff base derivative in ethanol (968, 1005, 1016, 1218, 1237, 1276, 1556, 1658 cm^{-1}) reveal that the 11-*cis* conformation is only slightly distorted from its solution conformation by the protein. The resonance Raman spectra of the protonated Schiff base of 9-*cis* retinal and isorhodopsin are also very similar. We have synthesized specifically deuterated retinals (C_{15}) to aid in the assignment of these spectra. The comparison of protonated Schiff base spectra of deuterated and undeuterated retinals shows that the 1240 cm^{-1} vibration in the unsubstituted molecules is sensitive to deuteration of the carbon and nitrogen in the Schiff base bond.

W-PM-H9 RESONANCE RAMAN SPECTROSCOPY OF CHEMICALLY MODIFIED RETINALS: ASSIGNING THE CARBON-METHYL VIBRATIONS IN THE RESONANCE RAMAN SPECTRUM OF RHODOPSIN. R. E. Cookingham*, A. Lewis and A. Kropf (Intr. by H. A. Scheraga), Cornell University, Ithaca, N.Y. 14853, and Amherst College, Amherst, Ma. 01002 (A.K.)

In order to assign the observed vibrational modes in the resonance Raman spectrum of the retinylidene chromophore of rhodopsin we have studied chemically modified retinals. One of the series of analogs investigated in this study is the n-butyl substituted retinals at C_9 and C_{13}. The results obtained for the 11-cis isomer have clearly assigned the C-CH_3 vibrational frequencies observed in the spectrum of the retinylidene chromophore [Lewis et al., J. Raman Spect. 1, 465 (1973); Callender, Biochemistry 15, 2621 (1976); Mathies et al., PNAS 73, 2169 (1976)]. The data on the 11-cis isomers of 9,13-di-n-butyl-retinal, 9-n-butyl, 13-methyl-retinal, and 13-n-butyl, 9-methyl-retinal show that the C_9-CH_3 vibration can be assigned to the vibrational mode observed in the 1017cm^{-1} region. The vibration detected at ~997cm^{-1} in the 11-cis isomer can be assigned to the C_{13}-CH_3 vibration. The C_5-CH_3 vibration is apparently not strongly resonance enhanced and thus does not contribute to the vibrations observed in this region. This is supported by data we have obtained on the des-methyl-retinal analogs. The splitting in the C_n-CH_3 (n=9,13) is characteristic of the 11-cis conformation. The results on the modified retinals do not support the hypothesis that the splitting arises from equilibrium mixtures of 11-cis, 12-s-cis and 11-cis, 12-s-trans in solution. Our data indicates that this assignment can be used to monitor local environmental and structural changes within the chromophore of bacteriorhodopsin and rhodopsin during proton pumping and visual excitation, respectively.

W-PM-H10 PRIMARY PHOTOCHEMICAL EVENTS IN VISUAL TRANSDUCTION: MODELING THE RESONANCE RAMAN SPECTRUM OF SQUID RHODOPSIN. M. Sulkes‡ A. Lewis and A.T. Lemley* (Intr. by B. M. Siegel), Cornell University, Ithaca, N.Y. 14853

We have obtained resonance Raman spectra of squid rhodopsin, isorhodopsin, bathorhodopsin and several other intermediates in the thermal sequence following the primary photochemical event. As in the case of bovine rhodopsin and isorhodopsin, many spectral features can be modeled successfully using 11-cis and 9-cis protonated Schiff bases, respectively [Mathies et al., J. Mol. Biol., in press]. We have also been able to model the spectrum of acid metarhodopsin (λ_{max}=500nm) with crystalline all-trans N-retinylidene-n-butylammonium hydrochloride (λ_{max}=431nm). These experimental results suggest that the color of visual pigments probably arises from excited state charge stabilization of the chromophore rather than ground state destabilization [Sulkes et al., PNAS, in press]. On the other hand, the spectrum of bathorhodopsin has not been successfully modeled. However, because the spectral features of the squid system at 77K allow for a greater batho contribution and because of the higher resolution of our spectra we have been able to identify several additional batho bands in frequency regions (e.g. 1147cm^{-1} and 1005cm^{-1}) where none were detected in the previously reported bovine spectra [Oseroff and Callender, Biochemistry 13, 4243 (1974)]. There appear to be considerable similarities in the spectral features of the batho intermediate in the region from 950cm^{-1} to 1700cm^{-1} for bovine, squid and bacterial rhodopsins we have studied. However the batho bacterial pigment does not exhibit any of the strong scattering observed below 950cm^{-1} in squid and bovine rhodopsin. Batho bacterial rhodopsin, unlike the analogous intermediate in the squid and bovine cases, does not originate from an 11-cis conformation. This could explain the lack of any low frequency vibrational modes in batho bacterial rhodopsin.

W-PM-H11 VISIBLE AND U.V. LINEAR DICHROISM OF RHODOPSIN, META I AND META II INTERMEDIATES IN RETINAL ROD OUTER SEGMENTS. Marc Chabre, Biophysique Moléculaire et Cellulaire, C.E.N.-G., F 38041 GRENOBLE, and Jacques Breton*, Biophysique, C.E.N.SACLAY, B.P.2, 91190 GIF SUR YVETTE.

The linear dichroism of R.O.S. chromophores has been studied from 220 to 650 nM with magnetically oriented macroscopic samples and a linear dichrograph based on the use of an elasto-optic modulator. Freshly isolated intact frog R.O.S. were suspended in Ringer in a standard spectrophotometer cell set in a 10 KGauss horizontal field perpendicular to the dichrograph beam. The apparatus measures directly $\varepsilon_{\parallel} - \varepsilon_{\perp}$ but does not provide a calibration. Effects of diffusion and form scattering were checked by glycerol imbibition.

In dark adapted R.O.S., beside the well known dichroism of the retinal chromophore α and β bands, a sharp negative peak is found at 295 nM, on the edge of the protein tryptophans absorpsion band, and a positive peak around 235 nM which may be related to the n_1-π transition in the helical backbone of the protein. Upon bleaching (10 sec., λ > 540 nM) at 2.5°C, Meta I and Meta II intermediates were isolated at different pHs. In both intermediates the retinal chromophore remains highly dichroic. In Meta II the chromophore main transition (380 nM) is even more constraint into the disc membrane plane than it is in native rhodopsin. Meta I - Meta II equilibrium, time evolution and effects of NH2OH have been studied. In the U.V., a small reduction of the 295 nM peak is observed when going to Meta I, and a larger one for Meta II, as well as a small increase of the 235 nM band.

W-PM-H12 PHYSICOCHEMICAL STUDIES ON BOVINE RHODOPSIN IN DETERGENT SOLUTIONS. Darrell R. McCaslin* and Charles Tanford, Department of Biochemistry, Duke University Medical Center, Durham, N.C. 27710.

Our approach to the study of the function and structure of a membrane bound protein is to solubilize the protein in a non-denaturing detergent solution in which it maintains its native function and presumably the structure which it had when associated with the membrane. The solubilized protein is then amenable to study by standard physicochemical techniques. We have initiated such a study of rhodopsin, the major membrane bound protein constituent of bovine rod outer segment disc membranes. Although it is generally accepted that rhodopsin is the primary photosensitive pigment, the mechanism by which it tranduces the absorption of a photon into a decreased sodium conductance across the rod outer segment plasma membrane is as yet unknown. Since the function of rhodopsin (aside from photon absorption) is unknown, we have utilized the regenerability of rhodopsin by 11-cis retinal as a criterion of nativity. By this criterion rhodopsin maintains its native state when solubilized in sodium cholate, Tween-80, octyl glucoside and octaethylene glycol n-dodecyl ether. Preliminary data indicate that the protein is oligomeric in each of these detergents, suggesting that the true native state is a species which is larger than a dimer. Upon bleaching, there are substantial changes in the sedimentation behavior which may indicate a change in the oligomeric state.

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W-PM-H13 PHASE SEPARATIONS IN RETINAL ROD OUTER SEGMENT MEMBRANE PHOSPHOLIPIDS. L.A. Sklar*, S. Bursten*, E.A. Dratz, Division of Natural Sciences, University of California, Santa Cruz, California, 95064

trans Parinaric acid (PnA, 9,11,13,15-all trans-octadecatetraenoic acid) is a fluorescent fatty acid of natural origin. Marked changes in the fluorescence intensity, lifetime, and polarization of PnA are observed in response to thermal phase transitions or lateral phase separations in several one or two component phospholipid dispersions. When PnA is added to a dispersion of phospholipids isolated from retinal rod outer segment membranes, the temperature dependence of the fluorescence intensity indicates lipid structural reorganization over the range of 20-27°. When the lipids are fractionated, the zwitterionic phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine, show a thermal transition over the range of 16-22° and the acidic phospholipids, phosphatidylserine (PS) and phosphatidylinositol show a transition below 15°. Of the zwitterionic phospholipids, PC alone shows a transition. Ca^{++} does not affect the transition of the zwitterionic phospholipids, but drastically reduces the magnitude of the transition of the acidic phospholipids without affecting its temperature. However, when Ca^{++} is added to a dispersion of the total phospholipid extract, the transition magnitude is reduced and the temperature range is lowered (from 20-27° to 15-22°). The transition which remains in the total phospholipids strongly resembles the transition of the zwitterionic phospholipids. These results are interpreted as a Ca^{++}-induced lateral phase separation of the acidic from the zwitterionic phospholipids in response to the chelation of Ca^{++} by PS. The Ca^{++} independent thermal transition is therefore interpreted as a PC lateral phase separation. The temperature dependence of the fluorescence polarization of PnA in disc membranes suggests a lateral phase separation of the components below 20°. The potential implications of these observations to visual excitation are considered.

W-PM-H14 PROTON AND PHOSPHOROUS NUCLEAR MAGNETIC RESONANCE STUDIES OF RHODOPSIN: EGG PHOSPHATIDYLCHOLINE RECOMBINANT MEMBRANES. D. F. O'Brien, N. Zumbulyadis, F. M. Michaels*, and R. A. Ott*, Eastman Kodak Research Laboratories, Rochester, New York, 14650.

Purified bovine rhodopsin was incorporated into phospholipid bilayers of egg phosphatidylcholine to yield recombinant membrane vesicles. Recombinants with molar ratios of 1:500, 1:200, and 1:100 rhodopsin to phosphatidylcholine were prepared. The recombinant membranes were sonicated to give single bilayer vesicles that range in size from 300 to 500Å in diameter as determined by negative-staining electron microscopy. The sonicated recombinants were examined by proton and phosphorous nuclear magnetic resonance spectroscopy. The chemical shifts of the phosphatidylcholine methyl, methylene, choline and vinyl protons are independent of the concentration of rhodopsin in the recombinant, whereas the linewidths of the methyl and methylene peaks increase with increasing rhodopsin concentration. Similarly the phosphorous signal due to the phosphate group is broadened as the rhodopsin content of the recombinant is increased. These data support the hypothesis that regions of the rhodopsin molecule are solvated by the hydrocarbon chains of the phospholipids, and that rhodopsin penetrates into the phospholipid bilayer membrane. We have demonstrated by the use of nuclear magnetic resonance shift and relaxation reagents that certain recombinant preparations are sealed in the dark. Some preliminary results on the effect of light on the observed nuclear magnetic resonance spectra will be described.

W-PM-H15 ISOLATION OF THREE ISOCHROMIC FORMS OF RHODOPSIN WHICH SHOW DIFFERENT BLEACHING KINETICS. H. Shichi, F. I. Hárosi*, S. Kawamura*, and T. Yoshizawa*, National Eye Institute and National Institute of Neurological and Communicative Diseases and Stroke, National Institutes of Health, DHEW, Bethesda, Maryland 20014, and Department of Biophysics, Kyoto University, Kyoto, Japan

The visual pigment rhodopsin, an integral membrane protein of the rod photoreceptor membranes, can be solubilized and purified only with detergents. Various workers have reported that rhodopsin in digitonin is decomposed by light through spectrally distinct intermediates with multiple rate constants for decay of each intermediate. Using ECTEOLA-cellulose and digitonin we have chromatographically purified rhodopsin and separated three rhodopsin fractions which are essentially identical in spectral properties. The first fraction(F-1) eluted with phosphate buffer(1 mM, pH 7.2) and the second fraction(F-2) eluted at higher ionic strength comprise more than 80% of the total rhodopsin loaded. F-1 and F-2 have regenerabilities of >80% and <30%, respectively. Bleaching intermediates (batho-, lumi-, and meta-rhodopsin I) formed by a flash bleach from F-1 and F-2 are indistinguishable in their thermal stability. Metarhodopsin I from F-2 decays faster than that from F-1. Metarhodopsin III formed from F-2 and F-3 also decays faster than that from F-1. Since partial delipidation of rod membranes prior to digitonin extraction results in an increase in F-1 with a concomitant decrease in F-2, the different bleaching kinetics observed for the different rhodopsin fractions may be related to the type and amount of phospholipid associated with opsin protein.

W-PM-H16 PROTEOLYTIC STUDIES ON THE AQUEOUS EXPOSURE OF FROG AND CATTLE RHODOPSIN. Gaw, J.E., E.A. Dratz, C.A. Vandenberg*, S.E. Slaughter*, E.A. Kelton*, M.J. Lipschultz*, and S. Schwartz, Div. of Natural Sci., Univ. of California, Santa Cruz, CA 95064.

Frog rhodopsin (rho) (apparent molecular weight by SDS-PAGE=39,500 daltons) is rapidly cleaved by subtilisin (1/3500, w/w) into two membrane bound fragments, F1 (27,400) and F2 (16,200). F1 is proteolyzed more slowly by subtilisin (1/35, w/w) into F1a (20,200) and F1b (11,000) which remain membrane bound. The rate of F1 (but not rho) proteolysis is greatly increased by the presence of antioxidants. Cattle rho (35,300) behaves much the same as frog except that in frog: a) F1 and F1a each lose about 1000 daltons by further proteolysis b) all fragments are slightly larger than in cattle (most of this difference is in F1) and c) the rate of F1 proteolysis is slightly faster than in cattle. Just prior to and concommitant with F1 and F2 formation rho is cleaved to form rho' (32,700) in cattle and at least two bands rho'(37,000) and rho" (35,000) in frog. In parallel with the formation of rho' F2 loses about 1000 daltons. Rho, the rho's, F1 and F1a bear carbohydrate. F2 bears the chromophore. In frog the total coomassie staining intensity decreases 25% $\pm$ 5% (S.E.M.) after extensive proteolysis, and during this period 16% $\pm$ 1% of the membrane amino acids are released. Simultaneously PAS staining decreases at most by 10% in both species. Since one of the two known carbohydrate moieties in cattle is penultimate to the N-terminus (P. Hargrave, pers. comm.), our results imply that the N-terminus of cattle remains intact and that rho is cleaved at the C-terminus. The spectrum and most of the regenerability remain intact. Transmission EM shows apparently closed vesicles before and after proteolysis. Freeze fracture EM reveals native membrane sidedness before proteolysis and predominantly native sidedness after proteolysis. Our results suggest that rho has at least three aqueous exposed regions widely spaced along its polypeptide chain. We thank D. Deamer for the freeze fracture EM.